# Orally Bioavailable Competitive CCR5 Antagonists

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The chemokine receptor CCR5 plays an important role in inflammatory and autoimmune disorders as well as in transplant rejection by affecting the trafficking of effector T cells and monocytes to diseased tissues. Antagonists of CCR5 are believed to be of potential therapeutic value for the disorders mentioned above and HIV infection. Here we report on the structure−activity relationship of a new series of highly potent and selective competitive CCR5 antagonists. While all compounds tested were inactive on rodent CCR5, this series includes compounds that cross-react with the cynomolgus monkey (cyno) receptor. One of these compounds, i.e., **26n**, has good PK properties in cynos, and its overall favorable profile makes it a promising candidate for in vivo profiling in transplantation and other disease models.

## Introduction

Chemokines comprise a family of molecules of about 80 amino acids that bind to G protein coupled heptahelical receptors (GPCR) on the cell surface. About 40 chemokines have been described to date (not including splice variants and viral analogues) and are classified into four families according to the spacing of key cysteins in the molecules. CC chemokines (with adjacent cysteins) and CXC chemokines (cysteins separated by one amino acid) are most abundant. One family member is known for C and CX3C chemokines each. Currently 11 CC and six CXC chemokine receptors are well-characterized. Engagement of chemokine receptors by their ligands triggers conformational changes in the receptors that lead to the initiation of a signaling cascade involving G protein binding, activation of kinases, $Ca^{2+}$ mobilization from intracellular stores, and cytoskeletal rearrangements, eventually leading to directed cell migration toward a concentration gradient of the respective ligand.[1]

A recent classification distinguishes between homeostatic chemokines/receptors (involved in the normal recirculation of lymphoid cells) and inflammatory chemokines/receptors. Inflammatory chemokines are expressed or upregulated in inflamed tissues and are responsible for the recruitment of effector cells, such as monocytes, memory/effector T cells, or granulocytes. Particularly among the inflammatory chemokines and their receptors, a certain redundancy is observed in that one chemokine can bind to several receptors and one receptor can bind several chemokines with high affinities. This pattern, as well as the concomitant expression of several chemokine receptors in one cell type, allows the very precise temporal and spatial regulation of cell trafficking.[1]

The CC chemokine receptor 5 (CCR5) is a representative of the inflammatory chemokine receptors. It is mainly expressed on effector/memory T cells, on monocytes and dendritic cells.[2] Its expression is upregulated by activation.[3] Several ligands can bind to CCR5 with high affinity (i.e., RANTES, MIP-1α, MIP-1*β*, MCP-2, HCC-1(9-74)). Among these, MIP-1*β* is the only selective CCR5 ligand. The CCR5 ligands are expressed by tissue cells and leukocytes upon cytokine stimulation and under inflammatory conditions.[1a,4] In addition to its role in inflammatory processes, CCR5 can function as a coreceptor for macrophage-infecting strains of HIV. A mutation in the human CCR5 gene (i.e. the CCR5Δ32 mutation), which leads to CCR5 deficiency in homozygous carriers, confers protection against HIV infection without being associated with increased morbidity.[1a,5] Various drug discovery programs have been initiated to identify small molecule CCR5 antagonists as anti-HIV agents.[6]

The currently most advanced CCR5 antagonist is Schering-Ploughs' development compound SCH-C (Figure 1).[7] It can be prepared in 13 steps involving equilibration and separation of a mixture of *E* and *Z* oxime isomers. It is highly potent, orally bioavailable, and selective for human CCR5 but does not cross-react with macaque CCR5.[7a] This is a surprising finding, because the sequences of human and cyno (cynomolgus) or rhesus monkey CCR5 are highly conserved.

Our interest in CCR5 was raised by a recently published report that highlights the role of CCR5 in human kidney allograft rejection.[8] Renal transplant recipients homozygous for CCR5Δ32 show significantly prolonged graft survival compared to CCR5-wild-type or heterozygous individuals. The role of CCR5 in transplantation was also confirmed in rodent models. As an example, CCR5 KO mice showed significantly prolonged graft survival in models of heterotopic heart[9,10] and islet[11] transplantation.

Here we report on the discovery of a new series of highly selective, readily available CCR5 antagonists that show good oral bioavailability. The compounds described here display cross-reactivity with cyno CCR5,

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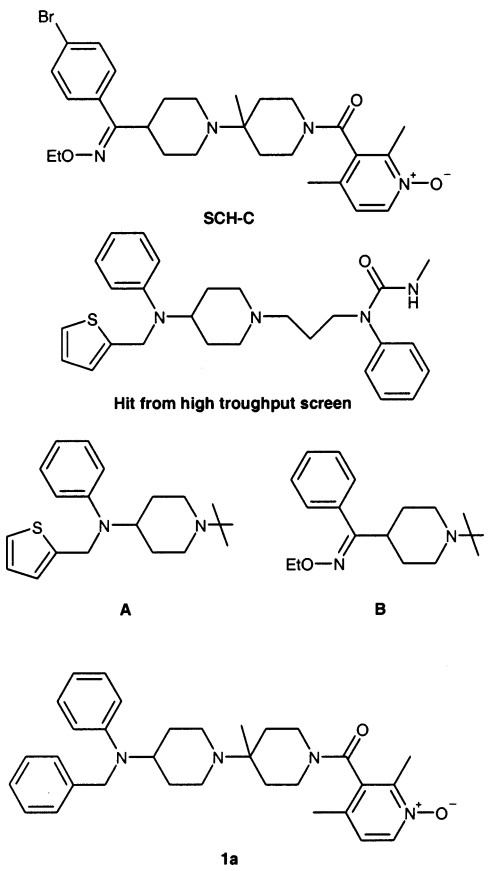
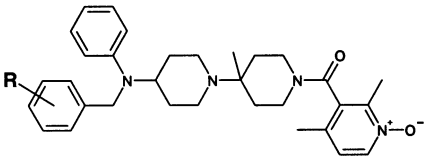



**Figure 1.** Design of the novel, highly potent CCR5 antagonist **1a**. HTS resulted in a hit ($IC_{50}$ value of <100 nM) containing fragment **A**, which was considered as a replacement for the phenylpiperidin-4-ylmethanone *O*-ethyloxime moiety (**B**) of **SCH-C**.

allowing further evaluation in primate disease models. The evaluation of the pharmacological properties of the compounds was performed in a series of in vitro assays including radioligand binding assays using membranes from CHO cells transfected with human, mouse, or cyno CCR5 and human MIP-1α as ligand. As functional in vitro readouts we determined the inhibition of agonist-induced $Ca^{2+}$ mobilization in CCR5-transfected CHO cells. Selected compounds were further profiled in cell migration assays with CCR5-transfected L1.2 cells or activated human peripheral blood lymphocytes.

High throughput screening of the Novartis compound collection resulted in a promising hit with an $IC_{50}$ value of 64 nM in the CCR5 ligand binding assay. The compound contained fragment **A**, which we considered to be a replacement for the phenylpiperidin-4-yl-methanone *O*-ethyloxime moiety (**B**) present in SCH-C. To probe our hypothesis, we prepared model compound **1a** (Figure 1). We were pleased to find **1a** to be a highly potent CCR5 antagonist exhibiting $IC_{50}$ values of 2.0, 14.9, and 14.9 nM in the binding, $Ca^{2+}$-mobilization, and migration assays, respectively (Table 1). Furthermore—and in contrast to SCH-C—compound **1a** cross-reacted with cyno CCR5 ($IC_{50}$ = 5.1 nM). It does not cross-react with mouse CCR5.[12] Encouraged by these findings, we studied the structure−activity relationship (SAR) and other properties of a series of related compounds.

**Table 1.** Modifications of the Benzyl Group



| | | $IC_{50}$ (nM)[a] binding | | | |
|---|---|---|---|---|---|
| compd | R | human | cyno | $Ca^{2+}$ mobil. | migration |
| 1a | H | 2.0 ± 0.8 | 5.1 ± 1.8 | 14.9 ± 2.9 | 14.9 ± 1.5 |
| 1b | 2-CN | 80 ± 2.9 | n.d.[b] | >3000 | n.d. |
| 1c | 3-CN | 0.6 ± 0.1 | 2.8 ± 0.1 | 10.8 ( 2.2 | n.d. |
| 1d | 4-CN | 3.8 ± 0.1 | 350[c] | 140[c] | n.d. |
| 1e | 3,4,5-OMe | >1000 | n.d. | n.d. | n.d. |

[a] Compounds tested at least three times; for details on the assays see the Experimental Section. [b] n.d., not determined. [c] Tested only once.

## Chemistry

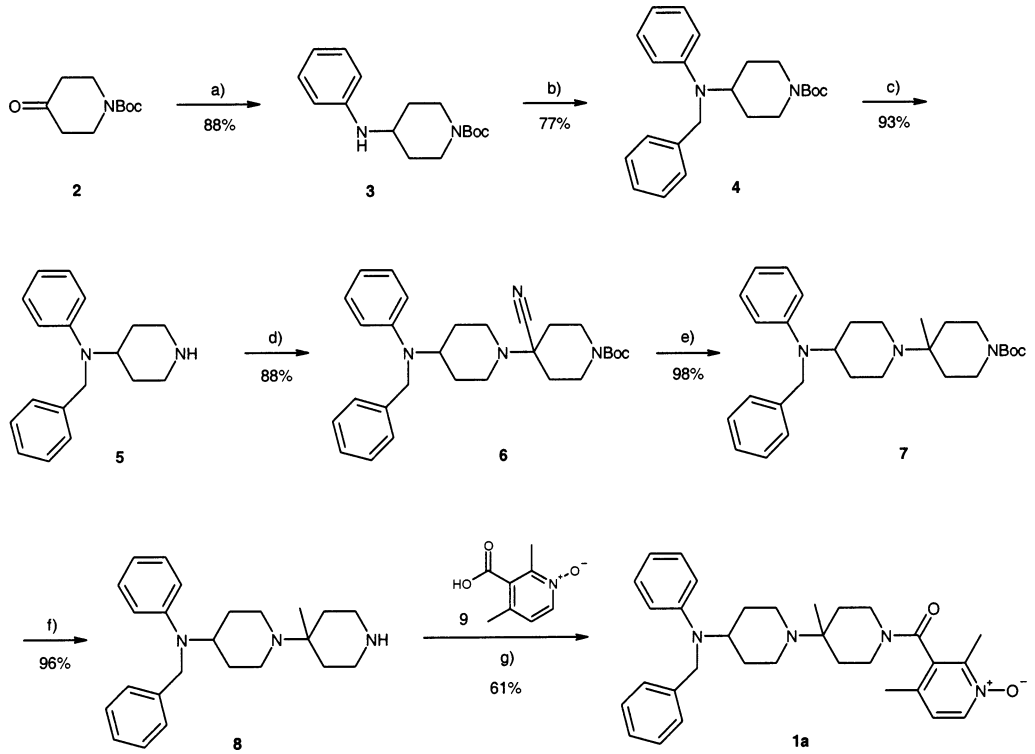
Compound **1a** was prepared from protected piperidone **2** in seven steps in 30% overall yield (Scheme 1). Reductive amination gave **3**, which was alkylated with benzyl bromide to yield **4**. Deprotection furnished **5**, which was reacted with piperidone **2** in the presence of $Ti(O\text{-}iPr)_4$ and subsequently treated with $Et_2AlCN$ to give nitrile **6**. Addition of excess quantities of $CH_3MgBr$ yielded **7**.[7c,13] Deprotection gave the free amine **8**, which was transformed into **1a** by condensation with benzoic acid derivative **9**.

Compounds **1b**−**e** were prepared from the intermediate **7** (Scheme 2). Hydrogenolytic removal of the benzyl protecting group (→**10**) was followed by treatment with the appropriately substituted benzyl bromide to give the compounds **11b**−**e**. Acidic removal of the protecting group yielded the advanced intermediates **12b**−**e**, which were transformed into the amides **1b**−**e**.

Several compounds with modifications in the left-hand portion were prepared (Table 2) starting with the orthogonally protected piperidones **13** and **14**, which were reacted with $Ti(O\text{-}iPr)_4$ and, subsequently, with $Et_2AlCN$ to give the intermediate **15** (Scheme 3). Treatment with $CH_3MgBr$ (→**16**) was followed by hydrogenolytic removal of the benzyl protecting group. The crude amine **17** was reacted with benzoic acid derivative **18** to give benzamide **19**. Acidic cleavage of the ketal yielded **20**. Reductive amination with the corresponding arylamine derivatives gave **21a**−**f**. Compound **22** was obtained by alkylation of **21a** with benzyl bromide, whereas benzylation of **21b** gave **23**. Compounds **24** and **25** were prepared from **20** by reductive amination with benzylamine and dibenzylamine, respectively.

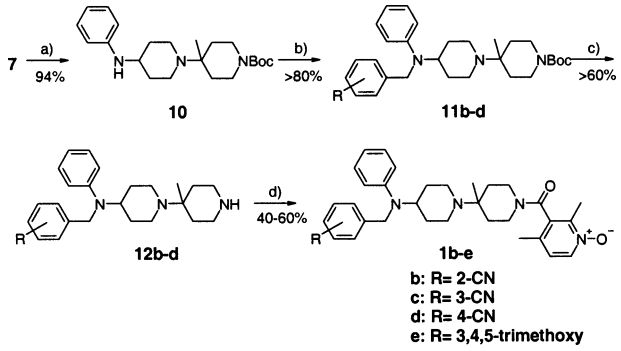
A number of derivatives with modifications in the right-hand portion (**26a**−**p**) were prepared from the advanced intermediate **27**, which is readily available from compound **3** (Scheme 4). Aminoarylation gave diphenylamino piperidine **28**, which was deprotected (→**29**) and reacted with $Ti(O\text{-}iPr)_4$ and, subsequently, with $Et_2AlCN$ to give **30**. Treatment with $CH_3MgBr$ (→**31**) followed by removal of the protecting group gave **27**. Condensation with the appropriate carboxylic acids yielded the compounds **26a**−**p** (Table 3). The sulfonamide **26q** was obtained from **27** and 2,4,6-trimethylbenzenesulfonyl chloride.

**Scheme 1.** Synthesis of Compound 1a[a]




[a] (a) Ph-$NH_2$, $(CH_3COO)_3NaBH$, AcOH, $(CH_2Cl)_2$; (b) Bn-Br, $K_2CO_3$, DMF; (c) TFA; (d) **2**, Ti(O-*i*Pr)$_4$, $(CH_2Cl)_2$, then add $Et_2AlCN$; (e) $CH_3MgBr$ (excess), THF; (f) TFA; (g) HBTU, DMF/EtN(*i*Pr)$_2$.

**Scheme 2.** Syntheses of Compounds **1b**−**e**[a]




[a] (a) $HCO_2NH_4$, Pd/C, $CH_3OH$; (b) (R)Bn-Br, $K_2CO_3$, NaI, DMF; (c) TFA; d) **9**, HBTU or EDC/HOBT, DMF/EtN(*i*Pr)$_2$.

Several compounds without the central methyl group of the bipiperidine core structure were prepared (Scheme 5 and Table 4). Compounds **32a**−**d** were obtained from **29**, which was reacted with the protected piperidone **2** to give **33**. Deprotection (→**34**) followed by amide formation gave the target molecules with the appropriate carboxylic acid gave **32a**−**d**.

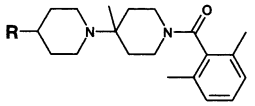
## Structure−Activity Relationship

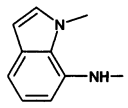
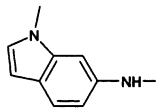
First we explored a few analogues of the highly potent CCR5 antagonist **1a** with a cyano substituent in different positions of the benzyl group (Table 1). The 3-substituted compound **1c** was found to be even more potent than unsubstituted **1a** on both human and cyno CCR5. The 2-substituted derivative **1b** was significantly less potent than **1a** in the human binding assay but highly inferior in the $Ca^{2+}$-mobilization assay. In addition, it was found to be almost inactive on cyno CCR5. The 4-substituted derivative **1d** was considerably less potent than **1c**. Compound **1e** with a trimethoxybenzyl group was found to be completely inactive. These findings suggest that substituents of the benzyl group are well-tolerated in the 3-position but can significantly reduce the affinity when attached to other ring positions. Furthermore, the substitution pattern seems to affect the reactivity on human vs cyno CCR5.

Next, we explored more pronounced changes of the left-hand portion (Table 2). To reduce the synthetic effort, we replaced the 2,4-dimethyl-1-oxy-nicotinamide residue of **1a** by a 2,6-dimethylbenzamide group and prepared compound **22**. The modification did not significantly alter the in vitro potency, as compounds **1a** and **22** showed comparable $IC_{50}$ values in the low nanomolar range in all four assays. Removal of the benzyl group resulted in a substantial loss in affinity (**21a**). Interestingly, substituents in the 4-position of the remaining phenyl ring such as Br, (**21b**), Cl (**21c**), and $CF_3$ (**21d**) compensated for this loss, whereas the biphenyl compound **21e** was considerably less potent than **21a**. The indole derivatives **21f** and **21g** showed potencies comparable to **21a**, indicating that substitution in the 2- and/or 3-position is tolerated. Removal of the phenyl group of compound **22** resulted in compound **24**, which did not inhibit CCR5 up to 1000 nM. Affinity for CCR5 was partly regained by the introduction of a second benzyl group (**25**). Very interestingly, compound **26a** with two phenyl groups was found to be as potent as **22**. Attempting to capitalize on the observation that **21b**, the 4-bromo derivative of **21a**, was about 50-fold more potent than **21a**, we prepared **23**, which is the 4-bromo derivative of **22**. It was found to be highly potent but, to our disappointment, not superior to **22**.

A broad variety of analogues of **26a** were prepared to explore the SAR of the right-hand side (Table 3).

**Table 2.** Modifications of the Left-Hand Portion



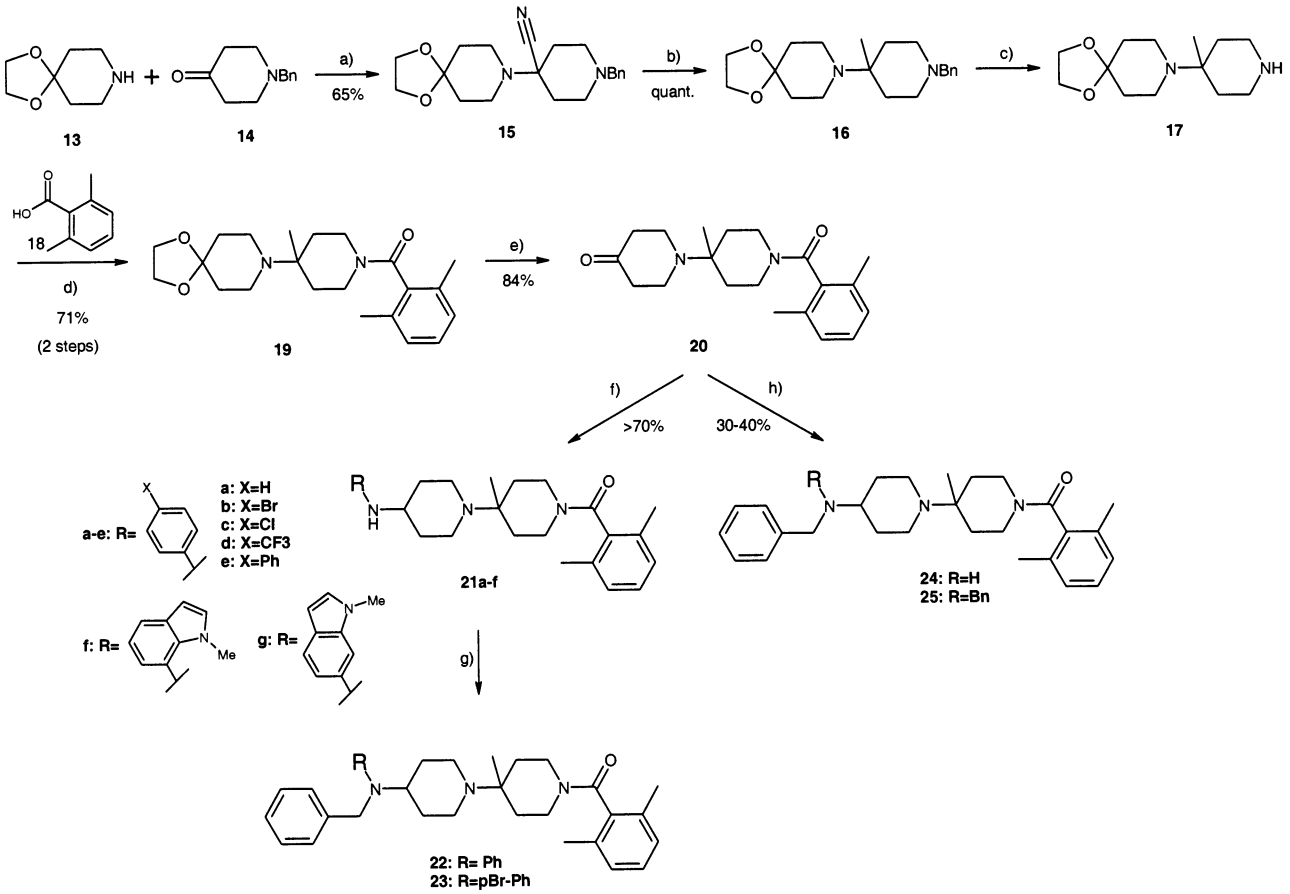
| Comp. | R- | binding (hu) $IC_{50}$ [nM][a] | binding (cy) $IC_{50}$ [nM][a] | $Ca^{2+}$-mobil. $IC_{50}$ [nM][a] | migration $IC_{50}$ [nM][a] |
|---|---|---|---|---|---|
| **21a** | PhNH- | 100 ± 17 | n.d. | n.d. | n.d. |
| **21b** | 4-BrPhNH- | 2.2 ± 0.1 | 0.5 ± 0.1 | 28.3 ± 6.3 | n.d. |
| **21c** | 4-ClPhNH- | 2.4 ± 0.1 | 2.0 ± 0.1 | 25.7 ± 4.2 | n.d. |
| **21d** | 4-($CF_3$)PhNH- | 0.8 ± 0.2 | 1.1 ± 0.4 | 27.3 ± 3.7 | n.d. |
| **21e** | 4-(Ph)PhNH- | 460[b] | n.d. | n.d. | n.d. |
| **21f** |  | 102 ± 5.5 | 147[b] | 2165 ± 455 | n.d. |
| **21g** |  | 91 ± 2.5 | 43[b] | 1680 ± 80 | n.d. |
| **22** | Ph,BnN- | 1.4 ± 0.3 | 3.0 ± 0.5 | 12.2 ± 2.2 | 14.4 ± 5.2 |
| **23** | *p*BrPh,BnN- | 2.3 ± 1.0 | 6.2 ± 0.1 | 15.6 ± 3.9 | n.d. |
| **24** | BnNH- | >1000 | n.d. | >1000 | n.d. |
| **25** | $Bn_2N$- | 75 ± 26 | n.d. | n.d. | n.d. |
| **26a** | $Ph_2N$- | 2.6 ± 1.5 | 0.7 ± 0.2 | 15.3 ± 1.3 | 14.4 ± 1.3 |

[a] Compounds tested at least three times; for details on the assays see the Experimental Section. [b] Tested only once.

Removal of one of the methyl substituents (**26b**) resulted in a decrease in affinity that is more pronounced for cyno CCR5 than for the human receptor, and removal of both methyl groups led to an even more significant decrease (**26c**). Surprisingly, compound **26d** with a single *N*,*N*-dimethylcarboxamide substituent in the 2-position turned out to be inactive up to 1000 nM. Compound **26e** containing an aliphatic six-membered ring instead of the aromatic ring was found to be only weakly active. More electron-donating methoxy substituents (**26f**) instead of methyl groups (**26a**) led to a loss in potency by a factor of 15, whereas two chlorine atoms (**26g**) did not cause significant changes. Interestingly, replacement of the 2,6-dimethylbenzamide by 1-naphthyl amide gave the highly potent compound **26h**. Two indole carboxamides were prepared with different attachment points of the indole residues. Compound **26i** linked via the six-membered ring was found to be 3−4-fold more potent than **26j**, which is linked via the five-membered ring. Pyridine and pyrimidine derivatives as well as their *N*-oxides (**26k**−**n**) proved to be highly potent inhibitors of both human and cyno CCR5. Substituents in the 2-position of the pyrimidine ring were found to be well-tolerated, as indicated by compounds **26o** and **26p**. Substitution of the carboxamide of **26a** by a sulfonamide resulted in the 15-fold less potent compound **26q**. For most compounds of this series we observed similar affinities to human and cyno CCR5.

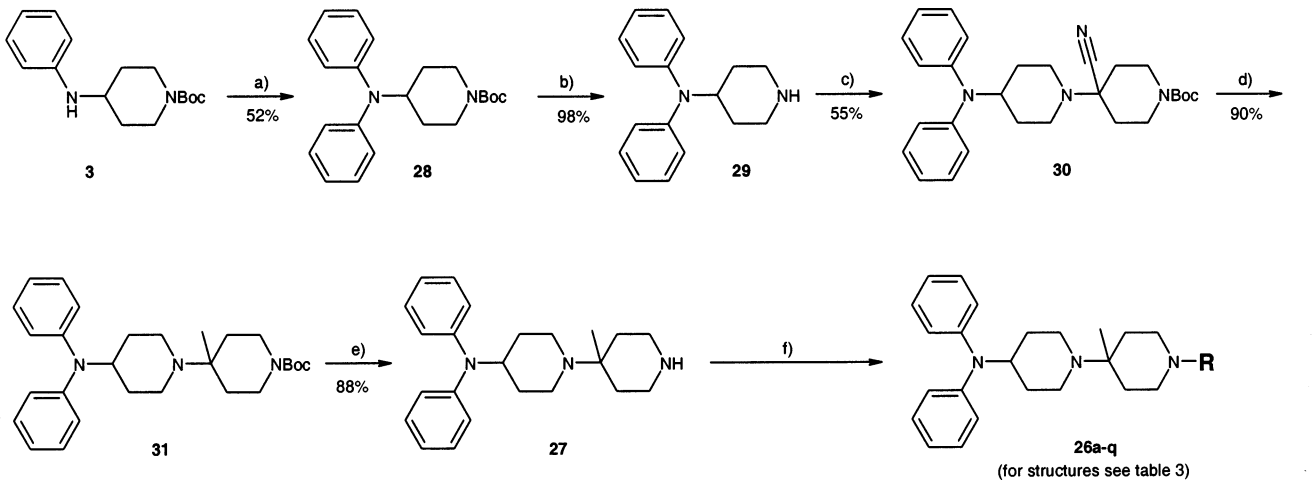
We also performed modifications of the central bipiperidine structure. Replacing the methyl substituent of the quaternary carbon atom by hydrogen gave readily available compound **32a**, which is less strained than **26a**. It was still active but 4-times less potent on human CCR5 and even more inferior on the cyno receptor. A few analogues of **32a** were prepared with more polar groups on the right-hand side. The pyridine derivative **32b** was as potent as **26a** (and its corresponding "methyl" derivative **26k**) on human CCR5 but slightly inferior in the cyno CCR5 binding assay. Compounds **32c** and **32d**, containing pyridine-*N*-oxide and pyrimidine, respectively, showed comparable potencies to **32a**

**Scheme 3.** Syntheses of Compounds **21**−**25**[a]




[a] (a) Ti(O-*i*Pr)$_4$, $(CH_2Cl)_2$, then add $Et_2AlCN$; (b) $CH_3MgBr$ (excess), THF; (c) $HCO_2NH_4$, Pd/C, $CH_3OH$; (d) HBTU, DMF/EtN(*i*Pr)$_2$; (e) dioxane, HCl; (f) corresponding aniline derivative, $(CH_3COO)_3NaBHNa$, $(CH_2Cl)_2$; (g) corresponding alkyl bromide, $K_2CO_3$, DMF; (h) corresponding amine, $(CH_3COO)_3NaBH$, AcOH, $(CH_2Cl)_2$.

**Scheme 4.** Syntheses of Compounds **26a**−**q**[a]




[a] (a) Iodobenzene, $Pd(OAc)_2$, BINAP, KO*t*Bu, toluene; (b) TFA; (c) Ti(O-*i*Pr)$_4$, $(CH_2Cl)_2$, then add $Et_2AlCN$; (d) $CH_3MgBr$ (excess), THF; (e) TFA; (f) amide coupling.

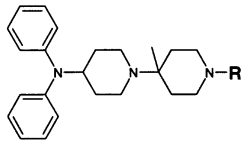
on human CCR5 but were considerably less potent on monkey CCR5.

## Evaluation of Activity

**Optimization of the $Ca^{2+}$-Mobilization Assay.** To evaluate the functional antagonism of CCR5 by compounds discussed here, we monitored transient rises in intracellular $Ca^{2+}$ levels triggered by CCR5 agonists. CCR5-transfected CHO cells were loaded with the $Ca^{2+}$-sensitive dye Fluo4, and the MIP-1α-induced transient increase of fluorescence indicative of increased intracellular $Ca^{2+}$ was monitored in a 384-well fluorescence image plate reader (FLIPR). None of the compounds tested induced $Ca^{2+}$ mobilization themselves, indicating that they do not act as agonists of CCR5 (data not shown).

Initial experiments in the FLIPR were conducted to monitor in the same experiment both potential agonism

**Table 3.** Modifications of the Right-Hand Portion



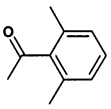
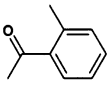
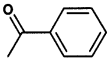
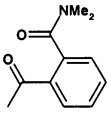
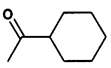
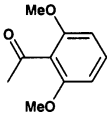
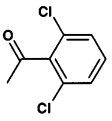
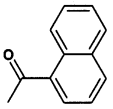
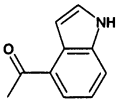
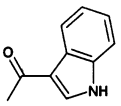
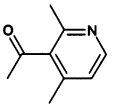
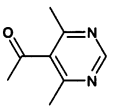
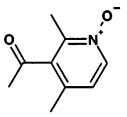
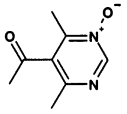
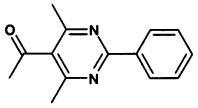
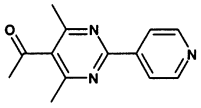
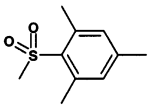
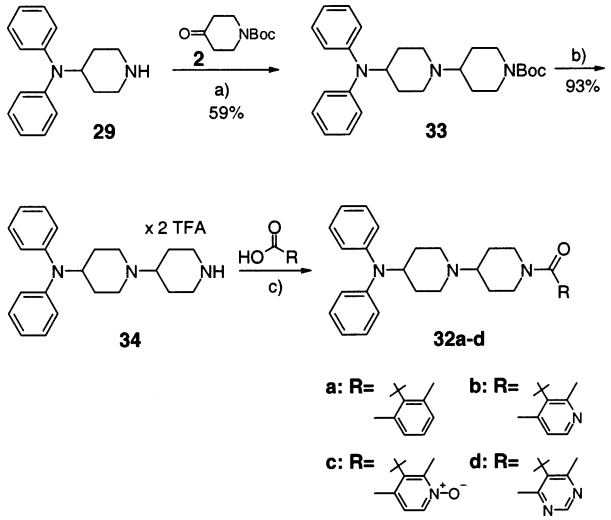
| Comp. | R | binding (hu) $IC_{50}$ [nM][a] | binding (cy) $IC_{50}$ [nM][a] | $Ca^{2+}$-mobil. $IC_{50}$ [nM][a] | migration $IC_{50}$ [nM][a] |
|---|---|---|---|---|---|
| **26a** |  | 2.6 ± 1.5 | 0.7 ± 0.2 | 15.3 ± 1.3 | 14.4 ± 1.3 |
| **26b** |  | 7.8 ± 4.2 | 28.6 ± 1.6 | 67 ± 41 | n.d. |
| **26c** |  | 200 ± 31 | 453[b] | 511 ± 206 | n.d. |
| **26d** |  | >1000 | n.d. | n.d. | n.d. |
| **26e** |  | 408 ± 70 | n.d. | 580[b] | n.d. |
| **26f** |  | 42 ± 24 | n.d. | n.d | n.d. |
| **26g** |  | 2.4 ± 1.2 | 3.5 ± 0.1 | 7.9 ± 2.7 | n.d. |
| **26h** |  | 3.7 ± 1.4 | 8.9 ± 1.7 | 29.4 ± 8.5 | n.d. |
| **26i** |  | 9.9 ± 0.1 | 31[b] | 102 ± 10 | n.d. |
| **26j** |  | 48 ± 3.5 | n.d. | 283 ± 30 | n.d. |
| **26k** |  | 2.3 ± 0.3 | 1.1 ± 0.3 | 11 ± 1.8 | n.d. |
| **26l** |  | 1.0 ± 0.4 | 2.2 ± 1.7 | 7.9 ± 2.0 | n.d. |

**Table 3** (Continued)

| | | | | | |
|---|---|---|---|---|---|
| **26m** |  | 3.3 ± 0.8 | 3.4 ± 0.4 | 16 ± 3.5 | n.d. |
| **26n** |  | 2.9 ± 1.5 | 12 ± 1.4 | 24 ± 0.2 | 13.3 ± 2.5 |
| **26o** |  | 8.5 ± 3.2 | 23 ± 7.5 | 31 ± 4.7 | 16.5 ± 2.2 |
| **26p** |  | 2.8 ± 1.1 | 7.9 ± 2.3 | 30 ± 5.7 | 24.6 ± 7.5 |
| **26q** |  | 41 ± 13 | n.d. | n.d. | n.d. |

[a] Compounds tested at least three times; for details on the assays see the Experimental Section. [b] Tested only once.

**Scheme 5.** Synteses of Compounds **32a**−**d**[a]

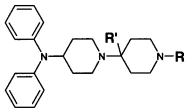



[a] (a) $(CH_3COO)_3NaBH$, AcOH, $(CH_2Cl)_2$; (b) TFA; (c) HBTU, DMF/EtN(*i*Pr)$_2$.

as well as antagonism of CCR5 by the compounds to be tested. The sequence of addition of the reagents was as follows: baseline fluorescence was monitored for 20 s, after which the compound to be tested was added at the desired concentration and fluorescence was further monitored for 2.5 min. Then the agonist was added and fluorescence was further recorded for 3 min. Inhibition of the MIP-1α-induced fluorescence by the compounds was used to calculate $IC_{50}$ values.

Under these experimental conditions we observed a poor correlation between the $IC_{50}$ values obtained in this assay and the radioligand binding assay, the discrepancy being most pronounced for lipophilic compounds with high log *P* values. Table 5 shows a series of highly

**Table 4.** Modifications of the Bipiperidine Core Structure



| Comp. | R- | R | binding (hu) $IC_{50}$ [nM][a] | binding (cy) $IC_{50}$ [nM][a] | $Ca^{2+}$-mobil. $IC_{50}$ [nM][a] |
|---|---|---|---|---|---|
| **26a** | $H_3C$- |  | 2.6 ± 1.5 | 0.7 ± 0.2 | 15.3 ± 1.3 |
| **32a** | H- |  | 11.1 ± 2.9 | 26[b] | 61 ± 21 |
| **32b** | H- |  | 3.0 ± 1.4 | 7.4 ± 3.6 | 15.5 ± 4.5 |
| **32c** | H- |  | 9.0 ± 1.0 | 117[b] | 127 ± 39 |
| **32d** | H- |  | 8.2 ± 1.7 | 62 ± 17 | 48 ± 17 |

[a] Compounds tested at least three times; for details on the assays see the Experimental Section. [b] Tested only once.

potent CCR5 inhibitors with $IC_{50}$ values in the range of 1−4 nM in the binding assay. Their $IC_{50}$ values in the $Ca^{2+}$-mobilization assay applying 2.5 min preincubation time were spread over a broad range from 1450 to 28 nM. Interestingly, the data from the $Ca^{2+}$-

**Table 5.** Effect of Preincubation Time on the $Ca^{2+}$-Mobilization Assay

| | $IC_{50}$ (nM) | | | |
|---|---|---|---|---|
| | | $Ca^{2+}$ mobil. | | |
| compd | binding (human) | 2.5 min | 2 h | cLogP[a] |
| 23 | 2.3 | 1450 | 28 | 7.8 |
| 26h | 3.7 | 610 | 29 | 6.8 |
| 22 | 1.4 | 200 | 12 | 6.8 |
| 26a | 2.6 | 250 | 15 | 6.4 |
| 26k | 2.3 | 114 | 11 | 5.3 |
| 26p | 2.8 | 125 | 25 | 4.9 |
| 26l | 1.0 | 28 | 8.0 | 4.4 |
| 26m | 3.3 | 33 | 16 | 3.0 |

[a] Calculated using Advanced Chemistry Development (ACD) Software Solaris V4.76 (© 1994−2003 ACD).

mobilization assay correlated with the lipophilicity of the compounds estimated by their cLogP values i.e., the more lipophilic the compound, the poorer its potency. We speculated that slow binding kinetics of the lipophilic compounds could account for the observed effects and studied the dependence of the measured $IC_{50}$ values on the preincubation time. Indeed, increasing the preincubation time in this assay yielded lower $IC_{50}$ values for lipophilic compounds, which now correlated well with the values obtained in the radioligand binding assay (Table 5). As a consequence, a preincubation time of 2 h was applied in both assay types.

**Further in Vitro Evaluation of Selected Compounds.** To assess the mode of inhibition of the compounds described in this report, we performed Schild−Gaddum analysis using the radioligand binding assay.[14] Concentration−response curves of the CCR5 agonist MIP-1α in the absence or presence of different concentrations of inhibitor were generated. With increasing antagonist concentrations we observed a shift of the concentration−response curves to the right in a parallel fashion without a significant decrease in $B_{max}$, which is indicative of a competitive mode of inhibition.

In addition to MIP-1α (which was used in our screening assays), other chemokines bind with high affinities to CCR5 and act as agonists, among them MIP-1*β*, RANTES, and HCC-1(9-74). We therefore addressed the question whether the compounds described here also inhibit $Ca^{2+}$ mobilization induced by these ligands. Several compounds were evaluated and found to antagonize all CCR5 ligands with similar potencies to MIP-1α. As an example, compound **26n** gave $IC_{50}$ values of 24, 16, 26, and 21 using MIP-α, MIP-1*β*, RANTES, or HCC-1(9-74), respectively.

For screening purposes, cells stably transfected with CCR5 were used. To confirm the antagonism of CCR5 in a "natural" cellular environment, we assessed their effect on the CCR5 ligand-induced migration of activated human peripheral blood lymphocytes (PBL). Prior to the assay, human peripheral blood mononuclear cells were activated by anti-CD3 and expanded in the presence of IL-2 for 14−21 days. It has been previously reported that under these conditions cells upregulate functional CCR5.[15] When tested in this assay, compound **26n** potently inhibited chemotaxis of human PBL induced by 10 nM MIP-1*β* with an $IC_{50}$ value of 5.6 ± 1.6 nM. When the chemokines MCP-1 or SDF-1 (which do not bind to CCR5) were used to induce migration, no inhibition of the migration was observed up to 300 nM, the highest concentration tested, demonstrating the selectivity of **26n**.

**Table 6.** PK Parameters of Selected Compounds (po administration)

| | AUC[a] (ng/mL h) | | $C_{max}$[b] (ng/mL) | | $T_{max}$ (h) | | MRT[c] (h) | | $F$[d] (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| compd | rat | cyno | rat | cyno | rat | cyno | rat | cyno | rat | cyno |
| 22 | 569 | 33 | 90 | 7 | 0.5 | 2.0 | 8.9 | 4.1 | 85 | 4 |
| 26a | 664 | | 81 | | 0.5 | | 6.9 | | 62 | 0 |
| 26h | 518 | | 70 | | 4.0 | | 14.4 | | 100 | |
| 26n | 1029 | 1594 | 165 | 91 | 0.5 | 4.0 | 5.1 | 6.0 | 94 | 58 |
| 26o | 234 | 115 | 30 | 12 | 1.0 | 4.0 | 6.8 | | 86 | 0 |
| 26p | 513 | | 73 | | 0.5 | | 5.8 | | 57 | |

[a] Area under the curve normalized to a dose of 1 mg/kg. [b] Maximal concentration normalized to a dose of 1 mg/kg. [c] Mean residence time. [d] Oral bioavailability obtained from ratio AUC (oral administration)/AUC (iv administration).

The compounds discussed here are selective antagonists of CCR5. Screening against other chemokine receptors such as CCR1, CCR2, CCR3, CCR4, CCR6, CCR7, CXCR1, CXCR2, CXCR3, CXCR4 and CXCR6 demonstrated no inhibitory activity of any of the compounds tested (i.e. **1a**, **22**, **26a**, **26h**, **26l**, **26m**, **26n**, **26o**, **26p**, **32a**) up to 1000 nM. Furthermore, screening against a broad panel of nonchemokine GPCRs (including muscarinic acetylcholine receptors) and ion channels did not show inhibition on any of these receptors or channels at 1000 nM.

The protein binding of selected compounds was assessed in human plasma. The free fractions of highly lipophilic compounds such as **22a**, **26a**, and **32a** were found to be below 3%. However, for more polar compounds, such as **26l**, **26m**, and **26n**, protein binding was not critical. The free fractions were determined to 14%, 23%, and 31%, respectively.

**In Vivo PK Properties.** The pharmacokinetic parameters of some of the compounds were determined in rats and in cynos (Table 6). The PK properties of these compounds were generally favorable in rats with oral bioavaiabilities of >50%. These properties were, however, not mirrored in cynos. Only **26n** gave a satisfactory PK profile in both rats and cynos (oral bioavailability of >50%, mean residence time 6 h), making it an interesting candidate for further in vivo evaluation.

## Rotamer Formation and Structural Analysis

For the series of CCR5 antagonists described here a sterically demanding amide-linked aromatic group attached to the bipiperidine core is crucial for high affinity binding to CCR5. However, this causes hindered rotation around two adjacent bonds, namely the amide bond (Figure 2, red) and the aryl bond (green). Compounds with nonsymmetrical aryl groups such as **26n** form four rotamers (two diastereomeric pairs of enantiomeric rotamers **PER1** comprising A and B and **PER2** comprising C and D; see Figure 2). Compounds with $C_2$ symmetrical aryl groups such as **26a** form only two enantiomeric rotamers, since a 180° rotation around the aryl bond does not cause any structural change (only A and B, because C is identical to B and D is identical to A). Accordingly, reversed-phase HPLC on an achiral phase (Merck, Chromolith Speedrod RP-18-e; 50 × 4.6 mm; $H_2O$/acetonitrile/TFA gradient) gave two signals for **26n** but only one signal for **26a**, whereas normal-

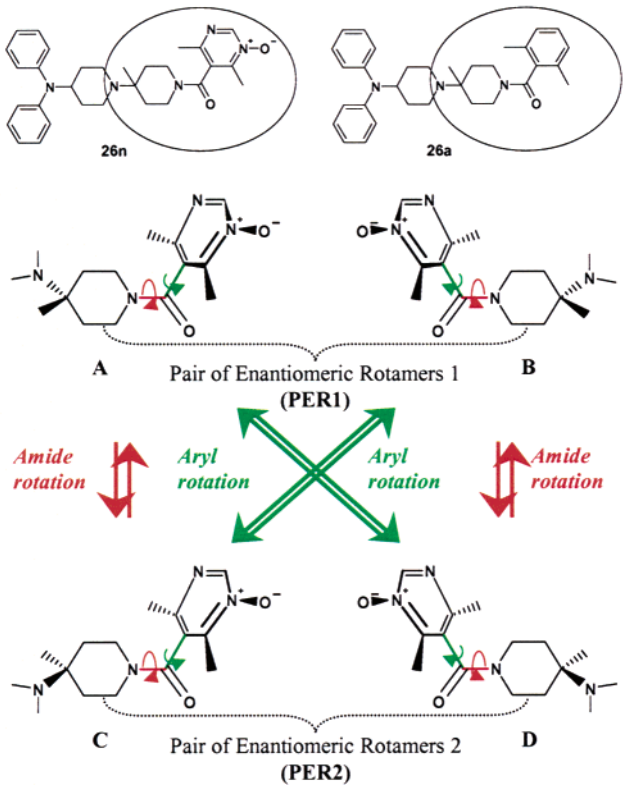



**Figure 2.** Rotamer formation due to hindered rotation around the amide bond (red) and the aryl bond (green). Compound **26n** forms two pairs of enantiomeric rotamers **PER1** (A and B) and **PER2** (C and D). **PER1** and **PER2** behave like diastereomers. Compounds with $C_2$ symmetrical aryl groups such as **26a** give rise to only two enantiomeric rotamers, because A becomes identical to D and B becomes identical to C.

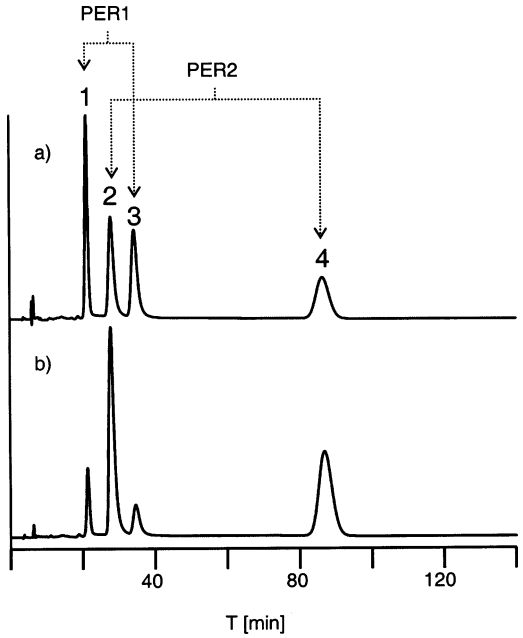



**Figure 3.** (a) HPLC trace of compound **26n** at equilibrium (~1:1:1:1 mixture of rotamers; conditions: CHIRALPAK AD-H, HPLC 150 × 2.1 mm, hexane/ethanol 15:85, 0.5 mL/min). (b) HPLC trace of freshly dissolved crystalline **26n**, which is enriched in the pair of enantiomeric rotamers **PER1** (~1:5:1:5 mixture of rotamers; see the X-ray structure analysis of **26n**; Figure 5 and Figure 2). **PER1**, giving rise to signals 2 and 4, comprises the enantiomeric rotamers A and B. A and B cannot be assigned. **PER2**, giving rise to signals 1 and 3, comprises the enantiomeric rotamers C and D.

phase HPLC on a chiral phase gave four peaks for **26n** (Figure 3a) and two signals for **26a**.

The $^1$H NMR spectrum of **26a** (one pair of enantimeric diastereomers) showed two distinct singlets for the remote diastereotopic methyl substituents of the $C_2$ symmetrical aryl group which, as expected, do not coalesce to a single signal, even at 150 °C. Contrary, the two *o*-methyl substituents of the nonsymmetrical aryl group of compound **26n** showed at equilibrium four singlets of equal intensity, indicating the presence of the two diastereomeric pairs of enantiomeric rotamers **PER1** and **PER2**. At 120 °C, four clearly separated signals were observed, and even at 150 °C, coalescence was not fully achieved, indicating a very high rotation barrier around the aryl bond (Figure 4a−c). The rotation around the aryl bond of compound **26b** with a nonsymmetrical aryl group containing only a single ortho-substituent was found to be significantly less hindered, as indicated by broad NMR signals at 25 °C and coalescence at 80−90 °C.

Crystals of compound **26n** suitable for X-ray analysis were obtained from ethyl acetate (Figure 5). The pair of enantiomeric rotamers **PER1** (A and B; Figure 2) with a 97.8° torsion angle for C11−C10−C12−O1 dominates in the crystalline state, but the observed electron density indicates the presence of the second pair of enantiomeric rotamers **PER2** (C and D, torsion angle C11−C10−C12−O1 = −82.2°). The ratio was determined to be approximately 9:1. Both piperidine rings that form the core structure of the molecule adopt chairlike conformations, with the central N-atom (N-6) being sp$^3$-hybridized (pyramidal) and the amide N-atom (N-5) being more sp$^2$-hybridized (planar). Interestingly, the rings are linked via an axial bond to N-6, whereas the methyl substituent (C37) occupies the equatorial position. NOE experiments confirmed the axial linkage of the piperidine rings also in solution.[14] We observed strong NOEs between the equatorial methyl substituent and both the axial and the equatorial H-atoms of the closest C-atoms of the other piperidine ring (Figure 5, green arrows). This indicates the equatorial position of the methyl substituent and, as a consequence, the dominance of the axial bond between the piperidine rings. An equatorial linkage would result in an axial methyl group that could only show NOEs to the equatorial but not to the axial H-atoms of the closest C-atoms. We believe that the axial linkage of the piperidine rings that form the core is crucial for high affinity binding to both human and cyno CCR5. Compounds of the "**26**-type" (with the methyl group) are more potent than compounds of the "**32**-type" (without the methyl group). In compounds **32a**−**d**, the piperidine rings are linked in an equatorial fashion, as indicated by NMR spectroscopy. In deuteriochloroform the central H-atom (replacing the methyl group of compounds **26**) gives rise to a triplet of triplets (chemical shift 2.44 ppm) with a large coupling constant ($J$ = 11.5 Hz) to the neighboring axial H-atoms and a small coupling constant ($J$ = 3.5 Hz) to the neighboring equatorial H-atoms. Thus, it occupies the axial position, and as a consequence, the bond between the piperidine rings must be equatorial. Presumably, the quaternary center of compounds **26**

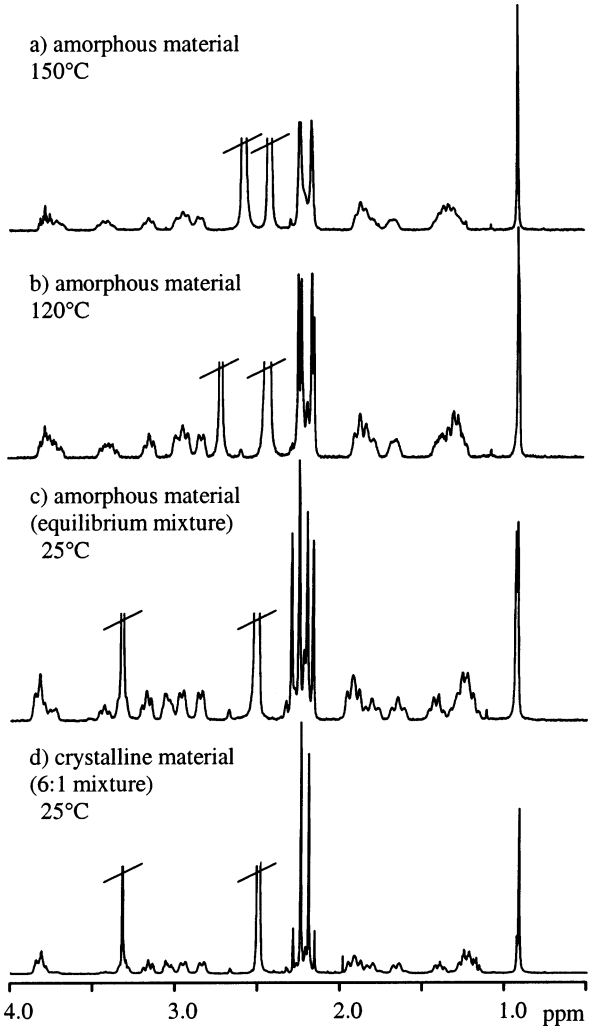



**Figure 4.** $^1$H NMR spectra of **26n**. Freshly dissolved amorphous material at 150 °C (a), 120 °C (b), and 25 °C, respectively. The rotamer ratio (**PER1**/**PER2**) was determined to ~1:1 on the basis of the intensities of the singlets at 2.29, 2.25, 2.20, and 2.16 ppm. Even at elevated temperatures, coalescence was not fully achieved, indicating a very high rotation barrier. (d) Freshly dissolved crystalline material recorded at 25 °C; the rotamer ratio was determined to ~6:1 on the basis of the intensities of the singlets at 2.25 and 2.20 ppm (**PER1**) and at 2.29 and 2.16 ppm (**PER2**).

contributes to the prevalence of the axial orientation in the solid state and, more importantly, in solution, leading to an improved preorganization of the bioactive conformation.

When crystalline **26n** was dissolved in DMSO (without elevating the temperature) and the NMR spectrum immediately recorded, a 6:1 mixture of two diastereomeric rotamers was observed (Figure 4d). NMR analysis of amorphous material showed a 1:1 mixture, representing the equilibrium ratio (Figure 4a). The time course of the equilibration was monitored by NMR in DMSO and 0.01 N HCl at 25 °C. Given the very high rotation barrier it was not surprising to find the equilibration to be a slow process in both solvents taking 16 h to reach the 1:1 ratio. At elevated temperatures, however, the equilibration was much faster (2 h at 37 °C; 0.5 h at 50 °C in 0.01 N HCl).

The HPLC analysis of the diastereomerically enriched crystalline material of **26n** on a chiral phase showed identical intensities for signals 1 and 3 and for signals 2 and 4, with signals 2 and 4 being 5-fold more intense. Thus signals 2 and 4 represent the enantiomers of **PER1** and signals 1 and 3 the enantiomers of **PER2** (see Figures 2 and 3). The enantiomeric rotamers cannot be assigned.

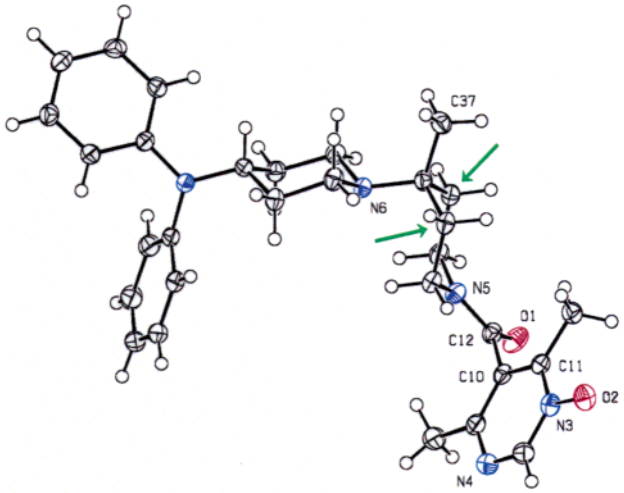



**Figure 5.** Structure of compound **26n** in the crystal[18] (atomic displacement ellipsoids drawn at the 50% probability level, hydrogen atoms drawn as spheres of arbitrary radius). One pair of enantiomeric rotamers **PER1** (A and B; see Figures 2 and 3) with torsion angle C11−C10−C12−O1 = 97.8° (as depicted) dominates, but the observed electron density indicates the presence of a second pair of enantiomeric rotamers **PER2** (C and D) with torsion angle C11−C10−C12−O1 = −82.2°. On the basis of the X-ray data, the ratio was determined to be approximately 9:1. The piperidine rings are linked via an axial bond to N6, whereas the methyl substituent (C37) occupies the equatorial position. In solution, strong NOE's between the methyl substituent and both the axial and the equatorial H-atoms of the closest C-atoms (green arrows) also indicate the dominance of the axial linkage of the piperidine rings.

Both amorphous **26n** (1:1 mix of **PER1** and **PER2**) and crystalline **26n** (6:1 mix of of **PER1** and **PER2**) were equally potent when freshly prepared solutions were tested (to avoid equilibration of the diastereomerically enriched material) in the binding assay as well as in the $Ca^{2+}$-mobilization assay.

Rotamer formation due to hindered rotation has also been reported for the structurally related compound SCH-C (Figure 1) and analogues thereof.[15] SCH-C coexists in solution as a 1:1:1:1 equilibrium mixture of four rotamers. As their equilibration is very slow, the rotamers could be separated on a preparative scale by HPLC using a chiral mobile phase. The individual rotamers were tested in an in vitro binding assay, and very interestingly, only one of them was found to be highly potent, whereas the second one was 8-fold and the two remaining ones 20-fold less potent.

## Conclusion

We have discovered a new series of highly potent and selective competitive antagonists of the chemokine receptor CCR5. Among them is compound **26n**, which is readily available and cross-reacts with cyno CCR5. Furthermore, it shows good PK properties in cynos, making it a promising candidate for in vivo profiling in transplantation and disease models.

## Experimental Section

**General.** All reactions were carried out under an atmosphere of dry argon. Commercially available absolute solvents were used. The NMR spectra were recorded on a Bruker Avance DPX 400 spectrometer. If not indicated otherwise, the spectra were recorded at ambient temperature. Compounds that form diastereomeric rotamers can give rise to complex spectra. In some cases only selected signals are listed in the Experimental Section. Copies of the spectra of all compounds are included in the Supporting Information. The MS spectra were obtained on a Finnigan MAT 90 mass spectrometer and the HR-MS spectra on a Finnigan MAT 900 S mass spectrometer.

**[4-(Benzylphenylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-yl](2,4-dimethyl-1-oxypyridin-3-yl)methanone (1a).** Carboxylic acid **9** (64.3 mg, 0.385) was dissolved in a mixture of DMF (2.5 mL) and diisopropyl ethylamine (DIPEA) (0.53 mL). HBTU (146 mg, 0.35 mmol) was added and the mixture was stirred for 20 min at 25 °C. Then compound **8** was added and stirring was continued for 16 h. The mixture was diluted with ethyl acetate and washed with $NH_4Cl$, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue subjected to flash chromatography ($SiO_2$, TBME → TBME/MeOH 10:1) to yield compound **1a** (120 mg, 61%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.18 (d, $J$ = 6.5 Hz, 1 H), 7.33−7.23 (m, 4 H), 7.22−7.18 (m, 2 H), 7.19 (t, $J$ = 7.5 Hz, 2 H), 6.66 (d, $J$ = 8.0 Hz, 2 H), 6.58 (t, $J$ = 7.0 Hz, 1 H), 4.47 (s, 2 H), 2.25, 2.20, 2.16 and 2.12 (4 s, 6 H), 0.92 and 0.91 (2 s, 3 H); HR-MS $[M + H]^+$ observed = 513.3227, estimated = 513.3230.

**2-({[1′-(2,4-Dimethyl-1-oxypyridine-3-carbonyl)-4′-methyl-[1,4′]bipiperidinyl-4-yl]phenylamino}methyl)-benzonitrile (1b).** A solution of 12b (77.6 mg, 0.20 mmol), 9 (66.8 mg, 0.40 mmol), HOBT (92 mg, 0.68 mmol), EDC (130 mg, 0.68 mmol), and DIPEA (1 mL) in DMF (1 mL) was stirred for 16 h at 25 °C. The mixture was diluted with ethyl acetate and washed with $NH_4Cl$, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue subjected to flash chromatography ($SiO_2$, TBME/cyclohexane 1:1 → TBME → TBME/MeOH 10:1) to yield compound 1b (46 mg, 43%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.17 (d, $J$ = 6.5 Hz, 1 H), 7.84 (d, $J$ = 7.5 Hz, 1 H), 7.60 (t, $J$ = 7.5 Hz, 1 H), 7.41 (t, $J$ = 7.5 Hz, 1 H), 7.37 (d, $J$ = 7.5 Hz, 1 H), 7.20 (m, 1 H), 7.13 (t, $J$ = 7.5 Hz, 2 H), 6.65 (m, 3 H), 4.61 (s, 2 H), 2.24, 2.20, 2.15 and 2.11 (4 s, 6 H), 0.91 and 0.90 (2 s, 3 H); HR-MS $[M + H]^+$ observed = 538.3185, estimated = 538.3182.

**3-({[1′-(2,4-Dimethyl-1-oxypyridine-3-carbonyl)-4′-methyl-[1,4′]bipiperidinyl-4-yl]phenylamino}methyl)-benzonitrile (1c)** was prepared from 10 by applying procedures as described for the synthesis of compound 1b: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.17 (d, $J$ = 6.5 Hz, 1 H), 7.67 (m, 2 H), 7.60 (d, $J$ = 7.5 Hz, 1 H), 7.52 (t, $J$ = 7.5 Hz, 1 H), 7.20 (m, 1 H), 7.11 (t, $J$ = 8.0 Hz, 2 H), 6.66 (d, $J$ = 8.0 Hz, 2 H), 6.61 (t, $J$ = 7.5 Hz, 1 H), 4.51 (s, 2 H), 2.24, 2.20, 2.15 and 2.11 (4 s, 6 H), 0.92 and 0.91 (2 s, 3 H); HR-MS $[M + H]^+$ observed = 538.3181, estimated = 538.3182.

**3-({[1′-(2,4-Dimethyl-1-oxypyridine-3-carbonyl)-4′-methyl-[1,4′]bipiperidinyl-4-yl]phenylamino}methyl)-benzonitrile (1d)** was prepared from 10 by applying procedures as described for the synthesis of compound 1b: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.18 (d, $J$ = 6.5 Hz, 1 H), 7.77 (m, 2 H), 7.46 (d, $J$ = 8.0 Hz, 2 H), 7.21 (m, 1 H), 7.11 (t, $J$ = 8.0 Hz, 2 H), 6.65 (d, $J$ = 8.0 Hz, 2 H), 6.61 (t, $J$ = 7.5 Hz, 1 H), 4.55 (s, 2 H), 2.25, 2.20, 2.17 and 2.12 (4 s, 6 H), 0.92 and 0.91 (2 s, 3 H); HR-MS $[M + H]^+$ observed = 538.3185, estimated = 538.3182.

**(2,4-Dimethyl-1-oxypyridin-3-yl){4′-methyl-4-[phenyl-(3,4,5-trimethoxybenzyl)amino]-[1,4′]bipiperidinyl-1′-yl}-methanone (1e)** was prepared from 10 by applying procedures as described for the synthesis of compound 1b: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.18 (d, $J$ = 6.5 Hz, 1 H), 7.19 (m, 1 H), 7.11 (t, $J$ = 8.0 Hz, 2 H), 6.68 (d, $J$ = 8.0 Hz, 2 H), 6.59 (t, $J$ = 7.5 Hz, 1 H), 6.56 (s, 2 H), 4.36 (s, 2 H), 3.68 (s, 6 H), 3.62 (s, 3 H), 2.26, 2.20, 2.16 and 2.11 (4 s, 6 H), 0.91 (s, 3 H); HR-MS $[M + H]^+$ observed = 603.3542, estimated = 603.3541.

**4-Phenylaminopiperidine-1-carboxylic Acid *tert*-Butyl Ester (3).** Sodium triacetoxyborohydride (55.1 g, 260 mmol) was added at 25 °C in four portions to a solution of **2** (50.0 g, 250 mmol), aniline (26.1 g, 280 mmol), and acetic acid (17.8 g, 296 mmol) in dichloroethane (400 mL), and the resulting mixture was stirred for 16 h. Sodium hydroxide (2 N) was added and the pH adjusted to 10. The mixture was extracted with $CH_2Cl_2$ and the organic phase dried with $Na_2SO_4$. The solvent was removed until precipitation started. Cyclohexane was added and the precipitated product **3** collected (54.6 g, 79%). An additional 6.2 g (9%) of **3** was obtained from the mother liquor: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.04 (t, $J$ = 8.5 Hz, 2 H), 6.57 (d, $J$ = 7.5 Hz, 2 H), 6.49 (t, $J$ = 7.5 Hz, 1 H), 5.43 (d, $J$ = 7.5 Hz, 1 H), 3.87 (m, 2 H), 3.30 (m, 1 H), 2.91 (m, 2 H), 1.88 (m, 2 H), 1.49 (s, 9 H), 1.22 (m, 2 H); MS ESI 277 $[M + H]^+$.

**4-(Benzylphenylamino)piperidine-1-carboxylic Acid *tert*-Butyl Ester (4).** A mixture of **3** (5.60 g, 20.0 mmol), $K_2CO_3$ (27.6 g, 200 mmol), benzyl bromide (34.2 g, 200 mmol), and DMF (150 mL) was heated at 100 °C for 16 h. The mixture was filtered, and the clear solution washed with sodium hydroxide (2 N), $NaHCO_3$, and brine and dried with $Na_2SO_4$. The solvent was removed, and the residue was suspended in TBME followed by the addition of cyclohexane. The precipitated product **4** (4.6 g, 63%) was filtered off. An additional 1.0 g (14%) of **4** was obtained from the mother liquor: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.25 (m, 4 H), 7.19 (t, $J$ = 7.0 Hz, 1 H), 7.11 (t, $J$ = 7.5 Hz, 2 H), 6.72 (d, $J$ = 7.5 Hz, 2 H), 6.61 (t, $J$ = 7.5 Hz, 1 H), 4.52 (s, 2 H), 2.88 (m, 2 H), 1.73 (m, 2 H), 1.48 (m, 2 H), 1.38 (s, 9 H); MS ESI 367 $[M + H]^+$.

**Benzylphenylpiperidin-4-ylamine (5).** A mixture of **4** (7.1 g, (19.4 mmol), TFA (20 mL), and water (2 mL) was stirred at 25 °C for 2 h. The pH was adjusted to 12 by addition of NaOH (4 N). The resulting suspension was filtered, and the solid residue was dissolved in ethyl acetate and extracted with NaOH (2 N). The organic phase was dried with $Na_2SO_4$ and the solvent removed until precipitation started. Cyclohexane was added and the precipitated product **5** was collected (4.80 g, 93%): $^1$H NMR (DMSO-$d_6$) $\delta$ 7.25 (m, 4 H), 7.19 (t, $J$ = 7.0 Hz, 1 H), 7.11 (t, $J$ = 7.5 Hz, 2 H), 6.72 (d, $J$ = 7.5 Hz, 2 H), 6.61 (t, $J$ = 7.5 Hz, 1 H), 4.52 (s, 2 H), 2.88 (m, 2 H), 1.73 (m, 2 H), 1.48 (m, 2 H), 1.38 (s, 9 H); MS ESI 267 $[M + H]^+$.

**4-(Benzylphenylamino)-4′-cyano-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (6).** A suspension of **5** (2.95 g, 11.0 mmol), **2** (2.20 g, 11.0 mmol), and titanium(IV) isopropoxide (3.13 g, 11.0 mmol) in 1,2-dichloroethane (50 mL) was stirred for 16 h at 25 °C. Diethylaluminum cyanide (22 mL, 1 M solution in toluene) was added and the mixture stirred for an additional 4 h. The reaction mixture was slowly quenched with $NaHCO_3$ and filtered. The clear solution was washed with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The solvent was evaporated to give compound **6** (4.8 g, 88%), which was used without further purification: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.25 (m, 4 H), 7.18 (t, $J$ = 7.0 Hz, 1 H), 7.09 (t, $J$ = 7.5 Hz, 2 H), 6.70 (d, $J$ = 7.5 Hz, 2 H), 6.58 (t, $J$ = 7.5 Hz, 1 H), 4.43 (s, 2 H), 3.87 (m, 1 H), 3.65 (m, 2 H), 3.20−3.02 (m, 4 H), 2.17 (m, 2 H), 2.07 (m, 2 H), 1.81 (m, 2 H), 1.63 (m, 4 H), 1.37 (s, 9 H); MS ESI 475 $[M + H]^+$.

**4-(Benzylphenylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (7).** At 0 °C, MeMgBr (12 mL, 3 M solution in ether) was slowly added to a suspension of **6** (3.4 g, 7.16 mmol) in THF (35 mL) and the resulting mixture stirred at 25 °C for 4 h. At 0 °C, $NH_4Cl$ (10% solution) was slowly added until gas evolution ceased. The mixture was extracted with ethyl acetate. The organic phase was washed with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The solvent was removed to give compound **7** (3.25 g, 98%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.25 (m, 4 H), 7.17 (t, $J$ = 7.0 Hz, 1 H), 7.07 (t, $J$ = 7.5 Hz, 2 H), 6.64 (d, $J$ = 7.5 Hz, 2 H), 6.56 (t, $J$ = 7.5 Hz, 1 H), 4.43 (s, 2 H), 3.86 (m, 1 H), 3.39 (m, 2 H), 3.15 (m, 2 H), 2.92 (m, 2 H), 2.17 (m, 2 H), 1.73 (m,

4 H), 1.55 (m, 2 H), 1.37 (s, 9 H), 1.23 (m, 2 H), 0.86 (s, 3 H); MS ESI 464 $[M + H]^+$.

**Benzyl(4′-methyl-[1,4′]bipiperidinyl-4-yl)phenylamine (8).** Compound **7** (1000 mg, 2.16 mmol) was added to TFA (6 mL) and water (0.2 mL) and the mixture stirred for 2 h at 25 °C. The pH was adjusted to 2−3 with NaOH (4 N). The mixture was extracted with $CH_2Cl_2$, and the organic phase was washed with $NaHCO_3$ and brine and then dried with $Na_2SO_4$. The solvent was removed to afford compound **8** (756 mg, 96%), which was used without further purification: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (m, 4 H), 7.18 (t, $J$ = 7.0 Hz, 1 H), 7.08 (t, $J$ = 7.5 Hz, 2 H), 6.64 (d, $J$ = 7.5 Hz, 2 H), 6.57 (t, $J$ = 7.5 Hz, 1 H), 4.43 (s, 2 H), 3.86 (m, 1 H), 2.96 (m, 2 H), 2.81 (m, 2 H), 2.50 (m, 2 H), 2.14 (m, 2 H), 1.86 (m, 2 H), 1.55 (m, 4 H), 1.32 (m, 2 H), 0.86 (s, 3 H); MS ESI 364 $[M + H]^+$.

**4′-Methyl-4-phenylamino-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (10).** A mixture of **7** (1000 mg, 2.16 mmol), $HCO_2NH_4$ (500 mg, 7.9 mmol), and $Pd(OH)_2$ (20%)/C (250 mg) in methanol (25 mL) was heated under reflux for 3 h. Following filtration the solvent was removed. The residue was suspended in ethyl acetate and washed with NaOH (1 N), $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$ and the solvent removed to give compound **10** (710 mg, 94%) as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.01 (t, $J$ = 7.5 Hz, 2 H), 6.52 (d, $J$ = 7.5 Hz, 2 H), 6.44 (t, $J$ = 7.5 Hz, 1 H), 5.31 (s, 2 H), 3.40 (m, 2 H), 3.21−3.05 (m, 3 H), 2.81 (m, 2 H), 2.12 (m, 2 H), 1.88 (m, 2 H), 1.73 (m, 2 H), 1.37 (s, 9 H), 1.37−1.28 (m, 4 H), 0.85 (s, 3 H); MS ESI 374 $[M + H]^+$.

**4-[(2-Cyanobenzyl)phenylamino]-4′-methyl-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (11b).** A suspension of **10** (150 mg, 0.40 mmol), 2-bromomethylbenzonitrile (196 mg, 1.00 mmol), $K_2CO_3$ (320 mg, 2.30 mmol), and NaI (110 mg, 0.73 mmol) in DMF (5 mL) was stirred for 16 h at 25 °C. The mixture was diluted with ethyl acetate and washed with $NaHCO_3$ and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue was subjected to flash chromatography ($SiO_2$, TBME/cyclohexane 1:9 → TBME) to yield compound **11b** (165 mg, 85%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.83 (d, $J$ = 7.5 Hz, 1 H), 7.60 (t, $J$ = 7.5 Hz, 1 H), 7.40 (m, 2 H), 7.12 (t, $J$ = 7.5 Hz, 2 H), 6.65 (m, 3 H), 4.60 (s, 2 H), 3.79 (m, 1 H), 3.40 (m, 2 H), 3.19 (m, 2 H), 2.95 (m, 2 H), 2.19 (m, 2 H), 1.76 (m, 4 H), 1.58 (m, 2 H), 1.37 (s, 9 H), 1.25 (m, 2 H), 0.87 (s, 3 H); MS ESI 489 $[M + H]^+$.

**2-{[(4′-Methyl-[1,4′]bipiperidinyl-4-yl)phenylamino]-methyl}benzonitrile (12b).** A solution of compound **11b** (165 mg, 0.34 mmol), TFA (2 mL), and water (0.2 mL) in $CH_2Cl_2$ (5 mL) was stirred was stirred for 2 h at 25 °C. The pH was adjusted to 11 with NaOH (4 N). The mixture was extracted with ethyl acetate, the organic phase was dried with $Na_2SO_4$, and the solvent removed to give compound **12b** (130 mg, quant) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.83 (d, $J$ = 7.5 Hz, 1 H), 7.60 (t, $J$ = 7.5 Hz, 1 H), 7.41 (t, $J$ = 7.5 Hz, 1 H), 7.36 (d, $J$ = 7.5 Hz, 1 H), 7.13 (t, $J$ = 7.5 Hz, 2 H), 6.65 (m, 3 H), 4.59 (s, 2 H), 3.77 (m, 1 H), 2.97 (m, 2 H), 2.83 (m, 2 H), 2.55 (m, 2 H), 2.15 (m, 2 H), 1.79 (m, 2 H), 1.62 (m, 2 H), 1.52 (m, 2 H), 1.34 (m, 2 H), 0.86 (s, 3 H); MS ESI 389 $[M + H]^+$

**1′-Benzyl-4-(2-methoxyethoxy)-[1,4′]bipiperidinyl-4′-carbonitrile (15).** A mixture of **13** (14.3 g, 100 mmol), **14** (19.0 g, 100 mmol), and titanium(IV) isopropoxide (28.4 g, 100 mmol) in 1,2-dichloroethane (200 mL) was stirred for 16 h at 25 °C. Diethylaluminum cyanide (200 mL, 1 M solution in toluene) was added, the mixture was stirred for an additional 3 h, and the temperature was kept below 45 °C. The reaction mixture was diluted with ethyl acetate (500 mL), slowly quenched with $NaHCO_3$, and filtered. The clear solution was washed with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The mixture was concentrated to a volume of 150 mL. Crystallization was initiated by adding cyclohexane. Compound **15** (22.0 g, 65%) was isolated as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.37−7.23 (m, 5 H), 3.88 (s, 4 H), 3.50 (s, 2 H), 2.80 (m, 2 H), 2.61 (m, 4 H), 2.15 (m, 4 H), 1.70−1.56 (m, 6 H); MS ESI 342 $[M + H]^+$

**1′-Benzyl-4-(2-methoxyethoxy)-4′-methyl-[1,4′]bipiperidinyl (16).** At 0 °C, MeMgBr (9.3 mL 3 M solution in ether) was slowly added to a mixture of **15** (1.9 g, 5.50 mmol) in THF (20 mL) and the resulting mixture stirred at 25 °C for 16 h. The suspension was diluted with ethyl acetate, and at 0 °C, $NH_4Cl$ (10% solution) was slowly added until gas evolution ceased. The mixture was extracted with ethyl acetate. The organic phase was washed with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The solvent was removed to give compound **16** (1.80 g, quant) as a slightly yellow oil, which was used without further purification: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.34−7.21 (m, 5 H), 3.85 (s, 4 H), 3.44 (s, 2 H), 2.48 (m, 2 H), 2.60 (m, 4 H), 2.20 (m, 2 H), 1.68 (m, 2 H), 1.57 (m, 4 H), 1.43 (m, 2 H), 0.83 (s, 3 H); MS ESI 331 $[M + H]^+$

**4-(2-Methoxyethoxy)-4′-methyl-[1,4′]bipiperidinyl (17).** A mixture of **15** (2.00 g, 6.05 mmol), $HCO_2NH_4$ (1.30 g, 20.6 mmol)k, and $Pd(OH)_2$ (20%)/C (0.80 g) in methanol (60 mL) was heated under reflux for 16 h. Following filtration, the solvent was removed. The residue was suspended in ethyl acetate and washed with NaOH (1 N), $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$ and the solvent removed to give crude compound **17** as a colorless solid, which was used without further purification: MS ESI 241 $[M + H]^+$.

**(2,6-Dimethylphenyl)-[4-(2-methoxyethoxy)-4′-methyl-[1,4′]bipiperidinyl-1′-yl]methanone (19).** Carboxylic acid **18** (1.03 g, 7.00 mmol) was dissolved in a mixture of DMF (10 mL) and diisopropyl ethylamine (7.8 mL). HBTU (2.60 g, 6.90 mmol) was added and the mixture was stirred for 15 min at 25 °C. Then crude compound **17** (6.05 mmol) was added and stirring was continued for 4 h. The mixture was diluted with ethyl acetate and washed with $NH_4Cl$, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue subjected to flash chromatography ($SiO_2$, TBME → TBME/MeOH 20:1) to yield compound **19** (1.60 g, 71%) as a slightly yellow oil: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.17 (t, $J$ = 7.5 Hz, 1 H), 7.07 (m, 2 H), 4.00 (m, 1 H), 3.86 (s, 4 H), 3.37 (m, 1 H), 3.20 (m, 1 H), 2.92 (m, 1 H), 2.48 (m, 4 H), 2.19 (s, 3 H), 2.14 (s, 3 H), 1.90 (m, 1 H), 1.73 (m, 1 H), 1.62 (m, 4 H), 1.38 (m, 1 H), 1.26 (m, 1 H), 0.90 (s, 3 H); MS ESI 373 $[M + H]^+$.

**1′-(2,6-Dimethylbenzoyl)-4′-methyl-[1,4′]bipiperidinyl-4-one (20).** A mixture of **19** (960 mg, 2.58 mmol), dioxane (30 mL), and HCl (6 N, 30 mL) was stirred at 25 °C for 16 h. Because **19** was not completely consumed, stirring was continued for 3 h at 50 °C. The mixture was diluted with ethyl acetate and washed with NaOH, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and compound **20** (710 mg, 84%) was isolated as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.16 (t, $J$ = 7.5 Hz, 1 H), 7.06 (m, 2 H), 4.02 (m, 1 H), 3.45 (m, 1 H), 3.26 (m, 1 H), 2.95 (m, 1 H), 2.79−2.67 (m, 4 H), 2.32 (m, 4 H), 2.18 (s, 3 H), 2.14 (s, 3 H), 1.94 (m, 1 H), 1.78 (m, 1 H), 1.43 (m, 1 H), 1.30 (m, 1 H), 0.91 (s, 3 H); MS ESI 329 $[M + H]^+$.

**(2,6-Dimethylphenyl)(4′-methyl-4-phenylamino-[1,4′]-bipiperidinyl-1′-yl)methanone (21a).** A mixture of **20** (674 mg, 2.05 mmol), acetic acid (246 mg, 4.1 mmol), $(CH_3COO)_3NaBH$ (478 mg, 2.26 mmol), aniline (210 mg, 2.26 mmol), and $(CH_2Cl)_2$ (20 mL) was stirred at 25 °C for 16 h. The mixture was diluted with ethyl acetate and washed with NaOH, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue was subjected to flash chromatography ($SiO_2$, TBME → TBME/MeOH 25:1) to yield compound **21a** (610 mg, 73%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.08−6.97 (m, 4 H), 6.55 (d, $J$ = 8.0 Hz, 2 H), 6.47 (t, $J$ = 7.0 Hz, 1 H), 5.34 (d, $J$ = 8.0 Hz, 1 H), 4.01 (m, 1 H), 3.34 (m, 1 H), 3.17 (m, 2 H), 2.91 (m, 2 H), 2.79 (m, 1 H), 2.17 (s, 3 H), 2.15 (m, 2 H), 2.12 (s, 3 H), 1.91 (m, 3 H), 1.74 (m, 1 H), 1.40−1.20 (m, 4 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 406.2860, estimated = 406.2858.

Compounds **21b**−**f** were prepared from **20** by following the procedure described for the synthesis of **21a**.

**[4-(4-Bromophenylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-yl](2,6-dimethyl-phenyl)methanone (21b)**: $^1$H NMR

(DMSO-$d_6$) $\delta$ 7.18−7.12 m, 3 H), 7.05 (m, 2 H), 6.51 (d, $J$ = 9.0 Hz, 2 H), 5.65 (d, $J$ = 8.0 Hz, 1 H), 4.01 (m, 1 H), 3.34 (m, 1 H), 3.17 (m, 2 H), 2.90 (m, 2 H), 2.79 (m, 1 H), 2.17 (s, 3 H), 2.15 (m, 2 H), 2.12 (s, 3 H), 1.91 (m, 3 H), 1.74 (m, 1 H), 1.40−1.20 (m, 4 H), 0.89 (s, 3 H); MS ESI 485/487 $[M + H]^+$

**[4-(4-Chloro-phenylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-yl](2,6-dimethylphenyl)methanone (21c)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (m, 4 H), 6.55 (d, $J$ = 8.5 Hz, 2 H), 5.61 (d, $J$ = 8.0 Hz, 1 H), 4.00 (m, 1 H), 3.34 (m, 1 H), 3.16 (m, 2 H), 2.90 (m, 2 H), 2.78 (m, 1 H), 2.17 (s, 3 H), 2.15 (m, 2 H), 2.12 (s, 3 H), 1.89 (m, 3 H), 1.75 (m, 1 H), 1.40−1.20 (m, 4 H), 0.89 (s, 3 H); HR-MS $[M + H]^+$ observed = 440.2467, estimated = 440.2469.

**(2,6-Dimethylphenyl)[4′-methyl-4-(4-trifluoromethylphenylamino)-[1,4′]bipiperidinyl-1′-yl]methanone (21d)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.33 (d, $J$ = 8.0 Hz, 2 H), 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (m, 2 H), 6.65 (d, $J$ = 8.5 Hz, 2 H), 6.21 (d, $J$ = 8.0 Hz, 1 H), 4.01 (m, 1 H), 3.38−3.13 (m, 3 H), 2.90 (m, 2 H), 2.80 (m, 1 H), 2.17 (s, 3 H), 2.17 (m, 2 H), 2.12 (s, 3 H), 1.91 (m, 3 H), 1.74 (m, 1 H), 1.42−1.20 (m, 4 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 474.2737, estimated = 474.2732.

**[4-(Biphenyl-4-ylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-yl](2,6-dimethylphenyl)methanone (21e)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.53 (d, $J$ = 7.5 Hz, 2 H), 7.42−7.33 (m, 4 H), 7.20 (t, $J$ = 7.5 Hz, 1 H), 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.06 (m, 2 H), 6.64 (d, $J$ = 9.0 Hz, 2 H), 5.62 (d, $J$ = 8.0 Hz, 1 H), 4.01 (m, 1 H), 3.34 (m, 1 H), 3.19 (m, 2 H), 2.92 (m, 2 H), 2.81 (m, 1 H), 2.19 (m, 2 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 1.94 (m, 3 H), 1.75 (m, 1 H), 1.42−1.20 (m, 4 H), 0.90 (s, 3 H); MS ESI 482 $[M + H]^+$.

**(2,6-Dimethylphenyl)[4′-methyl-4-(1-methyl-1*H*-indol-7-ylamino)-[1,4′]bipiperidinyl-1′-yl]methanone (21f)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (m, 3 H), 6.87 (d, $J$ = 8.0 Hz, 1 H), 6.77 (t, $J$ = 7.5 Hz, 1 H), 6.37 (d, $J$ = 8.0 Hz, 1 H), 6.32 (d, $J$ = 3.0 Hz, 1 H), 4.63 (m, 1 H), 4.08 (s, 3 H), 3.99 (m, 1 H), 3.42−3.15 (m, 3 H), 2.92 (m, 2 H), 2.81 (m, 1 H), 2.21 (m, 2 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 2.05−1.88 (m, 3 H), 1.74 (m, 1 H), 1.51 (m, 2 H), 1.38 (m, 1 H), 1.25 (m, 1 H), 0.91 (s, 3 H); HR-MS $[M + H]^+$ observed = 459.3121, estimated = 459.3124.

**(2,6-Dimethylphenyl)-[4′-methyl-4-(1-methyl-1*H*-indol-6-ylamino)-[1,4′]bipiperidinyl-1′-yl]methanone (21g)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.17 (m, 2 H), 7.05 (m, 2 H), 6.92 (d, $J$ = 3.0 Hz, 1 H), 6.44 (m, 2 H), 6.16 (d, $J$ = 3.0 Hz, 1 H), 5.06 (m, 1 H), 4.01 (m, 1 H), 3.61 (s, 3 H), 3.41−3.14 (m, 3 H), 2.92 (m, 2 H), 2.81 (m, 1 H), 2.21 (m, 2 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 2.04−1.88 (m, 3 H), 1.76 (m, 1 H), 1.42−1.20 (m, 4 H), 0.91 (s, 3 H); HR-MS $[M + H]^+$ observed = 459.3123, estimated = 459.3124.

**[4-(Benzylphenylamino)-4′-methyl-[1,4′]bipiperidinyl-1′-yl](2,6-dimethylphenyl)methanone (22).** A suspension of **21a** (142 mg, 0.35 mmol), $K_2CO_3$ (242 mg, 1.75 mmol), and benzyl bromide (600 mg, 3.5 mmol) in DMF (3 mL) was stirred at 100 °C for 16 h. Ethyl acetate was added and the mixture washed with $NaHCO_3$ and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue was subjected to flash chromatography ($SiO_2$, TBME/cycohexane 2:1 → TBME/cyclohexane 4:1) to yield compound **22** (115 mg, 66%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.32−7.23 (m, 4 H), 7.22−7.01 (m, 6 H), 6.66 (d, $J$ = 8.5 Hz, 2 H), 6.57 (t, $J$ = 7.0 Hz, 1 H), 4.45 (s, 2 H), 4.03 (m, 1 H), 3.77 (m, 1 H), 3.39 (m, 1 H), 3.17 (m, 1 H), 3.00 (m, 1 H), 2.89 (m, 2 H), 2.25−2.15 (m, 2 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 1.90−1.52 (m, 6 H), 1.37 (m, 1 H), 1.23 (m, 1 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 496.3329, estimated = 496.3328.

{**4-[Benzyl-(4-bromophenyl)amino]-4′-methyl-[1,4′]bipiperidinyl-1′-yl**}**(2,6-dimethylphenyl)methanone (23).** Compound **23** was prepared from **21b** by following the procedure described for the synthesis of **22**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.29 (t, $J$ = 7.5 Hz, 2 H), 7.25−7.18 (m, 5 H), 7.14 (t, $J$ = 7.5 Hz, 1 H), 7.04 (t, $J$ = 7.0 Hz, 2 H), 6.60 (d, $J$ = 9.0 Hz, 2 H), 4.45 (s, 2 H), 3.92 (m, 1 H), 3.75 (m, 1 H), 3.39 (m, 1 H), 3.16 (m, 1 H), 2.99 (m, 1 H), 2.88 (m, 2 H), 2.25−2.15 (m, 2 H), 2.16 (s, 3 H), 2.11 (s, 3 H), 1.86 (m, 1 H), 1.80−1.49 (m, 5 H), 1.36 (m, 1 H), 1.23 (m, 1 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 574.2436, estimated = 574.2433.

**(4-Benzylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)(2,6-dimethylphenyl)methanone (24).** A mixture of **20** (50 mg, 0.15 mmol), acetic acid (18 mg, 0.30 mmol), $(CH_3COO)_3NaBH$ (35 mg, 0.17 mmol), benzylamine (18 mg, 0.17 mmol), and $(CH_2Cl)_2$ (3 mL) was stirred at 25 °C for 16 h. The mixture was diluted with ethyl acetate and washed with NaOH, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue was subjected to flash chromatography ($SiO_2$, TBME/cyclohexane 4:1→ TBME) to yield compound **24** (19 mg, 30%) as a colorless oil: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.32 (d, $J$ = 7.0 Hz, 2 H), 7.29 (t, $J$ = 7.5 Hz, 2 H), 7.19 (t, $J$ = 7.5 Hz, 1 H), 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (m, 2 H), 3.98 (m, 1 H), 3.70 (s, 2 H), 3.37 (m, 1 H), 3.16 (m, 1 H), 2.93−2.67 (m, 3 H), 2.32 (m, 1 H), 2.17 (m, 1 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 2.06−1.63 (m, 7 H), 1.34 (m, 1 H), 1.20 (m, 1 H), 0.90 (s, 3 H); MS ESI 420 $[M + H]^+$.

**(4-Dibenzylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-(2,6-dimethylphenyl)methanone (25).** A mixture of **20** (82 mg, 0.25 mmol), acetic acid (30 mg, 0.50 mmol), $(CH_3COO)_3NaBH$ (117 mg, 0.55 mmol), dibenzylamine (54 mg, 0.28 mmol), and $(CH_2Cl)_2$ (5 mL) was stirred at 50 °C for 4 h. The mixture was diluted with ethyl acetate and washed with NaOH, $NaHCO_3$, and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue subjected to flash chromatography ($SiO_2$, TBME/cyclohexane 4:1→ TBME) to yield compound **25** (51 mg, 40%) as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.35 (d, $J$ = 7.0 Hz, 4 H), 7.29 (t, $J$ = 7.5 Hz, 4 H), 7.19 (t, $J$ = 7.5 Hz, 2 H), 7.14 (t, $J$ = 7.5 Hz, 1 H), 7.04 (m, 2 H), 3.92 (m, 1 H), 3.59 (s, 4 H), 3.37 (m, 1 H), 3.16 (m, 1 H), 3.00−2.81 (m, 3 H), 2.35 (m, 1 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 1.90−1.63 (m, 6 H), 1.58−1.43 (m, 2 H), 1.33 (m, 1 H), 1.20 (m, 1 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 510.3488, estimated = 510.3484.

**(2,6-Dimethylphenyl)(4-diphenylamino-4′-methyl-[1,4′]-bipiperidinyl-1′-yl)methanone (26a).** A mixture of compound **27** (250 mg, 0.71 mmol), carboxylic acid **9** (320 mg, 2.13 mmol), HBTU (568 mg, 1.50 mmol), DMF (5 mL), and diisopropyl ethylamine (DIPEA) (0.60 mL) was stirred for 16 h at 25 °C. The mixture was diluted with TBME and washed with NaOH and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue subjected to flash chromatography ($SiO_2$, TBME/cycylohexane 1:4 → TBME) to yield compound **26a** (240 mg, 70%) as a colorless foam: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (t, $J$ = 7.5 Hz, 4 H), 7.13 (t, $J$ = 7.5 Hz, 1 H), 7.03 (t, $J$ = 7.5 Hz, 2 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 3.78 (m, 2 H), 3.35 (m, 1 H), 3.00 (m, 2 H), 2.85 (m, 2 H), 2.21 (m, 2 H), 2.13 (s, 3 H), 2.04 (s, 3 H), 1.90 (m, 2 H), 1.77 (m, 1 H), 1.61 (m, 1 H), 1.37 (m, 1 H), 1.22 (m, 3 H), 0.90 (s, 3 H); HR-MS $[M + H]^+$ observed = 482.3171, estimated = 482.3171.

Compounds **26b**−**p** were prepared from **27** by following similar procedures as described for the synthesis of **26a**.

**(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-*o*-tolylmethanone (26b)**: $^1$H NMR (DMSO-$d_6$) (120°) $\delta$ 7.28−7.14 (m, 7 H), 7.08 (d, $J$ = 8.0 Hz, 1 H), 6.95 (t, $J$ = 7.5 Hz, 2 H), 6.84 (d, $J$ = 7.5 Hz, 4 H), 3.84 (m, 1 H), 3.45−3.26 (m, 4 H), 2.96 (m, 2 H), 2.27 (m, 2 H), 2.20 (s, 3 H), 1.94 (m, 2 H), 1.75 (m, 2 H), 1.38 (m, 4 H), 0.97 (s, 3 H); HR-MS $[M + H]^+$ observed = 468.3018, estimated = 468.3015.

**(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-phenylmethanone (26c)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.39 (m, 3 H), 7.33 (m, 2 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 8.0 Hz, 4 H), 3.86−3.70 (m, 2 H), 3.37−3.10 (m, 3 H), 2.92 (m, 2 H), 2.22 (m, 2 H), 1.92 (m, 2 H), 1.84−1.61 (m, 2 H), 1.42−1.15 (m, 4 H), 0.97 (s, 3 H); HR-MS $[M + H]^+$ observed = 454.2863, estimated = 454.2858.

**2-(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-carbonyl)-*N*,*N*-dimethylbenzamide (26d)**: $^1$H NMR (DMSO-$d_6$) (120°) $\delta$ 7.41 (m, 2 H), 7.27 (m, 6 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.85 (d, $J$ = 8.0 Hz, 4 H), 3.85 (m, 1 H), 3.41−3.22 (m, 3 H), 2.96 (m, 2 H), 2.86 (s, 6 H), 2.26 (m, 2 H), 1.94 (m, 2 H),

1.75 (m, 2 H), 1.43−1.28 (m, 5 H), 0.95 (s, 3 H); HR-MS [M + H]$^+$ observed = 525.3231, estimated = 525.3230.

**Cyclohexyl(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26e)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (t, $J$ = 8.0 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 3.81 (m, 1 H), 3.52 (m, 1 H), 3.13 (m, 1 H), 2.92 (m, 2 H), 2.21 (m, 2 H), 1.91 (m, 2 H), 1.72−1.53 (m, 8 H), 1.35−1.13(m, 11 H), 0.89 (s, 3 H); HR-MS [M + H]$^+$ observed = 460.3328, estimated = 460.3328.

**(2,6-Dimethoxyphenyl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26f)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.31−7.23 (m, 5 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 7.5 Hz, 4 H), 6.65 (dd, $J$ = 8.0/7.5 Hz, 2 H), 3.86−3.76 (m, 2 H), 3.72 (s, 3 H), 3.65 (s, 3 H), 3.14 (m, 1 H), 3.02 (m, 1 H), 2.97−2.81 (m, 3 H), 2.20 (m, 2 H), 1.90 (m, 2 H), 1.78 (m, 1 H), 1.65 (m, 1 H), 1.29−1.13 (m, 4 H), 0.88 (s, 3 H); HR-MS [M + H]$^+$ observed = 514.3072, estimated = 514.3070.

**(2,6-Dichlorophenyl)(4-diphenylamino-4′-methyl-[1,4′]-bipiperidinyl-1′-yl)methanone (26g)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.51 (t, $J$ = 8.0 Hz, 2 H), 7.42 (t, $J$ = 8.0 Hz, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 7.5 Hz, 4 H), 3.82 (m, 2 H), 3.25 (m, 1 H), 3.11 (m, 1 H), 2.98−2.81 (m, 3 H), 2.22 (m, 2 H), 1.97−1.80 (m, 3 H), 1.74 (m, 1 H), 1.33 (m, 2 H), 1.20 (m, 2 H), 0.90 (s, 3 H); HR-MS [M + H]$^+$ observed = 522.2078, estimated = 522.2079.

**(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-naphthalen-1-ylmethanone (26h)**: $^1$H NMR (DMSO-$d_6$) (120°, selected signals) $\delta$ 7.96−7.90 (m, 2 H), 7.75 (m, 1 H), 7.56−7.48 (m, 3 H), 7.38 (d, $J$ = 7.5 Hz, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 7.5 Hz, 4 H), 3.85 (m, 1 H), 2.26 (m, 2 H), 1.93 (m, 2 H), 0.96 (s, 3 H); HR-MS [M + H]$^+$ observed = 504.3022, estimated = 504.3015.

**(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-(1H-indol-4-yl)methanone (26i)**: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 11.27 (s, 1 H), 7.42 (d, $J$ = 8.0 Hz, 1 H), 7.38 (m, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 7.08 (t, $J$ = 8.0 Hz, 1 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.92 (t, $J$ = 7.0 Hz, 1 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 6.29 (m, 1 H), 3.81 (m, 2 H), 0.90 (s, 3 H); HR-MS [M + H]$^+$ observed = 493.2969, estimated = 493.2967.

**(4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)-(1H-indol-3-yl)methanone (26j)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 11.50 (s, 1 H), 7.62 (d, $J$ = 2.5 Hz, 1 H), 7.60 (d, $J$ = 8.0 Hz, 1 H), 7.40 (d, $J$ = 8.0 Hz, 1 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.12 (t, $J$ = 7.5 Hz, 1 H), 7.06 (t, $J$ = 7.5 Hz, 1 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 8.0 Hz, 4 H), 3.82 (m, 1 H), 3.61 (m, 2 H), 3.40 (m, 2 H), 2.95 (m, 2 H), 2.23 (m, 2 H), 1.92 (m, 2 H), 1.72 (m, 2 H), 1.37 (m, 2 H), 1.24 (m, 2 H), 0.92 (s, 3 H); HR-MS [M + H]$^+$ observed = 493.2966, estimated = 493.2967.

**(2,4-Dimethylpyridin-3-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26k)**: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.28 (m, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 7.09 (m, 1 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 8.0 Hz, 4 H), 3.81 (m, 1 H), 2.32, 2.22, 2.16 and 2.07 (4 s, 6 H), 0.92 and 0.91 (2 s, 3 H); HR-MS [M + H]$^+$ observed = 483.3128, estimated = 483.3124.

**(4,6-Dimethylpyrimidin-5-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26l)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.89 (d, $J$ = 5.0 Hz, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 7.5 Hz, 4 H), 3.81 (m, 2 H), 3.36 (m, 1 H), 3.10 (m, 1 H), 2.99−2.81 (m, 3 H), 2.33 (s, 3 H), 2.24 (s, 3 H), 2.22 (m, 2 H), 1.97−1.76 (m, 3 H), 1.65 (m, 1 H), 1.41 (m, 1 H), 1.32−1.16 (m, 3 H), 0.92 (s, 3 H); HR-MS [M + H]$^+$ observed = 484.3075, estimated = 484.3076.

**(2,4-Dimethyl-1-oxypyridin-3-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26m)**: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.16 (m, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 7.18 (m, 1 H), 6.97 (t, $J$ = 8.0 Hz, 2 H), 6.79 (d, $J$ = 8.0 Hz, 4 H), 3.82 (m, 1 H), 2.21, 2.13, 2.12 and 2.04 (4 s, 6 H), 0.91 (s (br), 3 H); HR-MS [M + H]$^+$ observed = 499.3078, estimated = 499.3073.

**(4,6-Dimethyl-1-oxypyrimidin-5-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26n)**: $^1$H NMR (DMSO-$d_6$) (selected signals) $\delta$ 8.96 (m, 1 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 8.0 Hz, 4 H), 3.82 (m, 1 H), 2.29, 2.25, 2.20 and 2.16 (4 s, 6 H), 0.92 and 0.91 (2 s, 3 H); HR-MS [M + H]$^+$ observed = 500.3031, estimated = 500.3026.

**(4,6-Dimethyl-2-phenylpyrimidin-5-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26o)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.38 (m, 2 H), 7.52 (m, 3 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 7.5 Hz, 4 H), 3.82 (m, 2 H), 3.40 (m, 1 H), 3.16 (m, 1 H), 2.98 (m, 2 H), 2.85 (m, 1 H), 2.42 (s, 3 H), 2.32 (s, 3 H), 2.23 (m, 2 H), 1.98−1.77 (m, 3 H), 1.67 (m, 1 H), 1.44 (m, 1 H), 1.35−1.18 (m, 3 H), 0.93 (s, 3 H); HR-MS [M + H]$^+$ observed = 560.3389, estimated = 560.3389.

**(4,6-Dimethyl-2-pyridin-4-ylpyrimidin-5-yl)(4-diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-yl)methanone (26p)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.76 (m, 2 H), 8.32 (m, 2 H), 7.27 (t, $J$ = 7.5 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 7.5 Hz, 4 H), 3.82 (m, 2 H), 3.40 (m, 1 H), 3.16 (m, 1 H), 2.98 (m, 2 H), 2.84 (m, 1 H), 2.44 (s, 3 H), 2.36 (s, 3 H), 2.23 (m, 2 H), 1.98−1.78 (m, 3 H), 1.66 (m, 1 H), 1.43 (m, 1 H), 1.35−1.17 (m, 3 H), 0.93 (s, 3 H); HR-MS [M + H]$^+$ observed = 561.3341, estimated = 561.3342.

**[4′-Methyl-1′-(2,4,6-trimethylbenzenesulfonyl)-[1,4′]bipiperidinyl-4-yl]diphenylamine (26q).** A mixture of compound **27** (70 mg, 0.20 mmol), 2,4,6-trimethylbenzenesulfonyl chloride (65 mg, 0.30 mmol), diisopropyl ethylamine (DIPEA) (0.50 mL), and $CH_2Cl_2$ (3 mL) was stirred for 4 h at 25 °C. The mixture was diluted with ethyl acetate and washed with $NaHCO_3$ and brine. The organic phase was dried with $Na_2SO_4$, the solvent was removed, and the residue was subjected to flash chromatography ($SiO_2$, TBME/cycylohexane 1:9 → TBME) to yield compound **26r** (65 mg, 61%) as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.04 (s, 2 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.79 (d, $J$ = 7.5 Hz, 4 H), 3.79 (m, 1 H), 2.94 (m, 4 H), 2.86 (m, 2 H), 2.48 (s, 6 H), 2.26 (s, 3 H), 2.18 (m, 2 H), 1.99 (m, 2 H), 1.75 (m, 2 H), 1.29 (m, 2 H), 1.16 (m, 2 H), 0.85 (s, 3 H); HR-MS [M + H]$^+$ observed = 532.2997, estimated = 532.2997.

**(4′-Methyl-[1,4′]bipiperidinyl-4-yl)diphenylamine (27).** A mixture of TFA (30 mL), water (1 mL), and **31** (5.50 g, 12.2 mmol) was stirred at 25 °C for 1 h. The pH was adjusted to 11 by slow addition of NaOH (4 M), and the mixture was extracted with ethyl acetate. The organic phase was dried with sodium sulfate and the solvent removed to give crude **27** (3.70 g, 88%) as a colorless solid, which was used without further purification: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.26 (t, $J$ = 7.5 Hz, 4 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.77 (d, $J$ = 8.0 Hz, 4 H), 3.81(m, 1 H), 2.85-(m, 6 H), 2.20 (t, $J$ = 6.5 Hz, 2 H), 1.91 (m, 2 H), 1.43 (m, 2 H), 1.29 (m, 2 H), 0.88 (s, 3 H); MS ESI 350 [M + H]$^+$.

**4-Diphenylaminopiperidine-1-carboxylic Acid *tert*-Butyl Ester (28).** A mixture of **3** (8.50 g; 30.8 mmol), iodobenzene (6.27 g; 30.8 mmol), $Pd(OAc)_2$ (0.29 g; 1.3 mmol), BINAP (0.83 g; 1.4 mmol), and t-BuOK (38.5 mL of 1 M solution in THF) in toluene (40 mL) was heated at 110 °C for 16 h. The mixture was diluted with ethyl acetate (100 mL), filtered, extracted with $NaHCO_3$ and brine, and dried with $Na_2SO_4$. The solvent was removed and the residue subjected to chromatography ($SiO_2$, TBME/cyclohexane 1:9→1:1) to give **28** (5.6 g, 52%) as a yellow solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (dd, $J$ = 8.0, 7.5 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.80 (d, $J$ = 8.0 Hz, 4 H), 4.09 (m, 1 H), 3.97 (m, 2 H), 2.88 (m, 2 H), 1.89 (m, 2 H), 1.32 (s, 9 H), 1.07 (m, 2 H); MS ESI 353 [M + H]$^+$.

**Diphenylpiperidin-4-ylamine (29).** A mixture of trifluoroacetic acid (90 mL), water (2.7 mL), and **28** (10.0 g; 28.4 mmol) was stirred for 3 h at 25 °C. The pH was adjusted to 11 by slow addition of NaOH (4 M), and the mixture was extracted with ethyl acetate. The organic phase was dried with sodium sulfate and the solvent removed to give **29** (7.0 g, 98%) as an oil, which was used without further purification: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.28 (dd, $J$ = 8.0, 7.5 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.80 (d, $J$ = 8.0 Hz, 4 H), 5.50−4.60 (m, 1 H), 4.02 (m, 1 H), 3.04 (m, 2 H), 2.75(m, 2 H), 1.89 (m, 2 H), 1.24 (m, 2 H); MS ESI 253 [M + H]$^+$.

**4′-Cyano-4-diphenylamino-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (30).** Titanium(IV) isopro-

poxide (7.87 g, 27.7 mmol) was added to a solution of **29** (7.00 g, 27.7 mmol) and **2** (5.52 g, 27.7 mmol) in $CH_2Cl_2$ (25 mL), and the mixture was stirred for 16 h at 25 °C and then for 16 h at 20 °C. Diethylaluminum cyanide (55.4 mL, 1 M solution in toluene) was slowly added and stirring continued for 3 h at 25 °C. The mixture was quenched by slow addition of $NaHCO_3$ and subsequently diluted with ethyl acetate. After filtration, the solution was extracted with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The residue was dissolved in ether. Addition of cyclohexane gave **30** (7.1 g, 55%) as a colorless solid: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.28 (t, $J$ = 8.0 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.82 (d, $J$ = 8.0 Hz, 4 H), 3.94 (m, 1 H), 3.68 (m, 2 H), 3.13 (m, 2 H), 3.00 (m, 2 H), 2.19 (m, 2 H), 2.08 (m, 2 H), 1.98 (m, 2 H), 1.53 (m, 2 H), 1.37 (s, 9 H), 1.25 (m, 2 H).

**4-Diphenylamino-4′-methyl-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (31).** At 0 °C, MeMgBr (26.5 mL, 3 M solution in ether) was slowly added to a mixture of **30** (7.00 g, 15.2 mmol) in THF (60 mL) and the resulting mixture stirred at 25 °C for 2 h. The suspension was diluted with ethyl acetate and at 0 °C $NH_4Cl$ (10% solution) was slowly added until gas evolution ceased. The mixture was extracted with ethyl acetate. The organic phase was washed with $NaHCO_3$ and brine and dried with $Na_2SO_4$. The solvent was removed and the residue crystallized from ether/hexane to give compound **31** (6.10 g, 90%): $^1$H NMR (DMSO-$d_6$) $\delta$ 7.26 (t, $J$ = 8.0 Hz, 4 H), 6.96 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 3.79 (m, 1 H), 3.30 (m, 2 H), 3.10 (m, 2 H), 2.89 (m, 2 H), 2.19 (m, 2 H), 1.89 (m, 2 H), 1.64 (m, 2 H), 1.35 (s, 9 H), 1.22 (m, 4 H), 0.87 (s, 3 H); MS ESI 450 $[M + H]^+$.

Compounds **32a**−**d** were prepared from **34** by following similar procedures as described for the synthesis of **26a**.

**(2,6-Dimethylphenyl)(4-diphenylamino-[1,4′]bipiperidinyl-1′-yl)methanone (32a)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (t, $J$ = 7.5 Hz, 2 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 7.5 Hz, 4 H), 4.58 (m, 1 H), 3.82 (m, 1 H), 3.17 (m, 1 H), 2.94 (m, 1 H), 2.85 (m, 2 H), 2.73 (m, 1 H), 2.44 (m, 1 H), 2.28 (m, 2 H), 2.16 (s, 3 H), 2.08 (s, 3 H), 1.91−1.78 (m, 3 H), 1.62 (m, 1 H), 1.35−1.14 (m, 4 H); HR-MS $[M + H]^+$ observed = 468.3020, estimated = 468.3015.

**(2,4-Dimethylpyridin-3-yl)(4-diphenylamino-[1,4′]bipiperidinyl-1′-yl)methanone (32b)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.30 (m, 1 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.11 (m, 1 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 4.56 (m, 1 H), 3.82 (m, 1 H), 3.16 (m, 1 H), 2.99 (m, 1 H), 2.86 (m, 2 H), 2.77 (m, 1 H), 2.45 (m, 1 H), 2.34, 2.27, 2.18, 2.11 (4 s, 6 H), 2.28 (m, 2 H), 1.91−1.79 (m, 3 H), 1.64 (m, 1 H), 1.39−1.16 (m, 4 H); HR-MS $[M + H]^+$ observed = 469.2972, estimated = 469.2967.

**(4,6-Dimethylpyrimidin-5-yl)(4-diphenylamino-[1,4′]-bipiperidinyl-1′-yl)methanone (32c)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.91 (s, 1 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.15 (t, $J$ = 7.5 Hz, 1 H), 7.05 (t, $J$ = 7.5 Hz, 2 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 7.5 Hz, 4 H), 4.53 (m, 1 H), 3.82 (m, 1 H), 3.22 (m, 1 H), 3.03 (m, 1 H), 2.91−2.77 (m, 3 H), 2.46 (m, 1 H), 2.35 (s, 3 H), 2.29 (m, 2 H), 2.27 (s, 3 H), 1.91−1.79 (m, 3 H), 1.65 (m, 1 H), 1.40−1.16 (m, 4 H); HR-MS $[M + H]^+$ observed = 470.2921, estimated = 470.2920.

**(2,4-Dimethyl-1-oxypyridin-3-yl)(4-diphenylamino-[1,4′]-bipiperidinyl-1′-yl)methanone (32d)**: $^1$H NMR (DMSO-$d_6$) $\delta$ 8.18 (d, $J$ = 6.5 Hz, 1 H), 7.27 (t, $J$ = 8.0 Hz, 4 H), 7.20 (m, 1 H), 6.97 (t, $J$ = 7.5 Hz, 2 H), 6.78 (d, $J$ = 8.0 Hz, 4 H), 4.51 (m, 1 H), 3.82 (m, 1 H), 3.27 (m, 1 H), 3.00 (m, 1 H), 2.89−2.75 (m, 3 H), 2.46 (m, 1 H), 2.28 (m, 2 H), 2.23, 2.17, 2.16, 2.08 (4 s, 6 H), 1.91−1.79 (m, 3 H), 1.65 (m, 1 H), 1.39−1.16 (m, 4 H); HR-MS $[M + H]^+$ observed = 485.2925, estimated = 485.2917.

**4-Diphenylamino-[1,4′]bipiperidinyl-1′-carboxylic Acid *tert*-Butyl Ester (33).** Sodium triacetoxyborohydride (1.0 g, 4.7 mmol) was slowly added at 25 °C to a solution of **29** (1.06 g, 4.4 mmol), **2** (1.00 g, 5.0 mmol), and acetic acid (0.62 g, 10.33 mmol) in dichloroethane (15 mL), and the resulting mixture was stirred for 4 h at 65 °C. Sodium hydroxide (2 N) was added and the pH adjusted to 10. The solvent was removed and the residue subjected to chromatography ($SiO_2$, TBME/cyclohexane 1:9→ TBME) to give **33** (1.06 g, 59%) as a colorless solid. $^1$H NMR ($CDCl_3$) $\delta$ 7.25 (d, $J$ = 8.0/7.5 Hz, 4 H), 6.98 (t, $J$ = 7.5 Hz, 2 H), 6.82 (d, $J$ = 8.0 Hz, 4 H), 4.28−4.00 (m, 2 H), 3.82 (m, 1 H), 2.96 (m, 2 H), 2.66 (m, 2 H), 2.42−2.27 (m, 3 H), 1.99 (m, 2 H), 1.74 (m, 2 H), 1.52−1.32 (m, 4 H), 1.44 (s, 9 H); MS ESI 436 $[M + H]^+$.

**[1,4′]Bipiperidinyl-4-yldiphenylamine (34).** A solution of **33** (1.06 g, 2.40 mmol), TFA (2.5 mL), and water (0.1 mL) in $CH_2Cl_2$ (5 mL) was stirred at 25 °C for 4 h. The mixture was slowly added to diethyl ether and the precipitate collected. Compound **34** (1.25 g, 93%) was isolated as a ditriflouroacetate: $^1$H NMR (DMSO-$d_6$/$D_2O$) $\delta$ 7.29 (t, $J$ = 8.0 Hz, 4 H), 7.01 (t, $J$ = 7.5 Hz, 2 H), 6.81 (d, $J$ = 8.0 Hz, 4 H), 4.25 (m, 1 H), 3.42 (m, 5 H), 3.21 (m, 2 H), 2.89 (m, 2 H), 2.13 (m, 4 H), 1.74 (m, 2 H), 1.55 (m, 2 H); MS ESI 336 $[M + H]^+$.

**Crystal Structure Determination and Refinement of 26n.** Diffraction data were collected at 100 K with a Bruker AXS SMART 6000 CCD detector on a three-circle platform goniometer with Cu Kα radiation from a rotating anode generator equipped with Osmic multilayer mirrors. A semiempirical absorption correction was applied, based on the intensities of symmetry-related reflections measured at different angular settings.[19] The structure was solved and refined on $F^2$ with the SHELXTL suite of programs.[20] Non-hydrogen atoms were refined with anisotropic displacement parameters, and hydrogen atoms were calculated in idealized positions and refined using a riding model. A significant residual electron density peak (1.07 e·Å$^3$ 1.22 Å away from N4) indicated a rotational disorder of ring C10−C11−N4−C8−N3−C9 around the C10−C12 bond. The disorder was modeled by refining two different orientations of O2 (O2 and O2b) with a 0.893(3) (a) to 0.107 (b) distribution.

Final data: $C_{30}H_{37}N_5O_2$, $M_r$ = 499.65, triclinic, space group $P-1$ (No. 2) with $a$ = 8.183(2) Å, $b$ = 10.587(2) Å, $c$ = 16.792(3) Å, α = 99.40(1)°, $\beta$ = 97.45(1)°, $\gamma$ = 110.97(1)°, $V$ = 1311.9(5) Å$^3$, $Z$ = 2, $D_c$ = 1.265 g·cm$^{-3}$, 17 779 reflections measured, 3869 independent, 2.72° < $\theta$ < 60.79°, $T$ = 100(2) K, 348 parameters, 13 restraints, $R_1$ = 0.0328, $wR_2$ = 0.0790 for 3563 reflections with $I > 2\sigma(I)$, $R_1$= 0.0356, $wR_2$ = 0.0812 for all 3869 data, GoF = 1.067, restrained GoF = 1.082, res el dens = +0.21/−0.20 e·Å$^3$.

Crystallographic data (excluding structure factors) for **26n** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC-221017.

## In Vitro Assays. CCR5 Ligand Binding Assay.

Membranes were prepared from CHO cells transfected with human or cyno CCR5. Cells were homogenized using a Polytron in 20 mM HEPES pH 7.4, 1 mM EDTA, including protease inhibitors. Membranes were centrifuged at 28 000*g*, resuspended in fresh buffer, and stored frozen at −80 °C in aliquots. $^{125}$I-Labeled MIP-1α (2000 Ci/mmol) was purchased from Amersham Biosciences. The scintillation proximity technology was used for all binding assays. In brief, in a 96-well microtiter plate (Opti-plate, Packard) WGA-coated SPA beads (1 mg/well in 50 μL assay buffer) were mixed with 50 μL of membranes containing 7−15 μg total protein and 50 μL of compound or 100 nM unlabeled MIP-1α or assay buffer. Finally, 50 μL of $^{125}$I-labeled MIP-1α in assay buffer was added (final concentration 20−25 pM). The composition of the assay buffer was 50 mM HEPES, 6 mM $CaCl_2$, 12 mM $MgCl_2$, 100 mM NaCl, and 0.5% BSA (protease inhibitors). The plate was then sealed and incubated under constant shaking for 2 h at room temperature. After centrifugation (200*g* for 10 min) to sediment the SPA beads, the plate was measured using a TopCount (Packard), and the data were analyzed using GraphPad Prism software (3.0).

**$Ca^{2+}$-Mobilization Experiments.** CCR5-transfected CHO cells were detached from the culture plates and loaded in suspension for 1 h at 37 °C with 4 μM Fluo4 (Molecular Probes) in Hank's balanced salt solution containing 20 mM HEPES pH 7.4, 0.1% BSA, and 2.5 mM probenecid. Cells were washed twice and distributed to microtiter plates. Unless stated

otherwise, compounds were added to cells and incubated for 2 h at room temperature. The cell suspension was then transferred to 384-well black microtiter plates and placed in a FLIPR (Molecular Devices) and baseline fluorescence was recorded for 20 s. MIP-1α was added to a final concentration of 10 nM and fluorescence was continuously recorded over 3 min. $IC_{50}$ values were derived from the concentration-dependent reduction of the difference $F_{max} - F_{min}$, where $F_{max}$ is the maximal fluorescence intensity in the presence of the agonist and $F_{min}$ is the minimal (=baseline) fluorescence prior to agonist addition. In some experiments, RANTES, MIP-1$\beta$, or HCC-1(9-74) were used instead of MIP-1α.

**Cell-Migration Experiments.** Cell migration (chemotaxis) was assessed in the transwell assay in which the migration of the cells across a porous membrane along a positive chemokine gradient is tested. In brief, $5 \times 10^5$ cells in 100 $\mu$L medium were added to Costar Transwell inserts (pore size 5 $\mu$m). Chemokines were added to the lower compartment of the transwell chamber in a volume of 600 $\mu$L. The inserts containing the cells were placed above the lower compartment and incubated at 37 °C for 4 h (when CCR5 transfected L1.2 cells were used) or 90 min (when human PBL were used). After this incubation time, cells that had migrated into the lower compartment were counted by acquiring all events in a flow cytometer (FACS Calibur) for 30 s. Compounds to be tested as chemokine receptor antagonists were placed at the same concentration in both the upper and the lower compartment, to avoid the formation of a compound gradient across the membrane. $IC_{50}$ values were derived from the concentration-dependent inhibition of the chemokine-induced cell migration.

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**Supporting Information Available:** Copies of the $^1$H NMR spectra of all compounds described in the Experimental Section and details on the X-ray analysis of **26n**. This material is available free of charge via the Internet at http://pubs.acs.org.

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